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Phylogeography of Two Loligo Squid (Cephalopoda: Myopsida) in the Gulf of Mexico and the Northwestern Atlantic Ocean.

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PHYLOGEOGRAPHY OF TWO *LOLIGO* SQUID (CEPHALOPODA: MYOPSIDA) IN THE GULF OF MEXICO AND THE NORTHWESTERN ATLANTIC OCEAN

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
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Doctor of Philosophy

in

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by
Scott William Herke
B.S., Louisiana State University, 1986
M.S., University of Maine, 1988
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DEDICATION

My family has always demonstrated to me the important values in life. No matter how busy he was, my father (William H. Herke, Ph.D.) believed in making time to spend with his family, and he put tremendous effort into taking us on adventures in the wilderness. So, I spent much of my childhood fishing on the Gulf of Mexico, hunting ducks in the coastal marshes of Louisiana, canoeing rivers in the state, or traveling to other parts of the country to go hiking. My mother (Joan F. Herke, RN,C) put her own career on hold to take care of the family while my siblings and I were growing up. We could count on her to help us with our trials and tribulations; further, she let us know that we were capable of doing anything we wished to do. Both of my parents let us know that they loved us. With regard to my siblings, my brothers David and John as well as my sister Kristin have tried to smooth my path over the years, and David has been especially concerned for my welfare. Finally, Linda M. Heffernan has been a wonderful wife and friend to me. Her love and support has made completing this project possible, and I hope we get to do more fun activities together now that we are both finishing our doctoral degrees.

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The initial identified specimens of *Loligo pealei* and *L. plei* were graciously provided by Harriet Perry and Guillermo Sánchez (Gulf Coast Research Laboratory, Ocean Springs, Mississippi); further, Harriet tutored me regarding the identification of the two species by morphological characters. Additional specimens were collected primarily by the National Marine Fisheries Service (NMFS) and the South Carolina Department of Natural Resources (SCDNR). Victor Nordahl (NMFS, Northeast Fisheries Science Center, Woods Hole, Massachusetts) as well as Charles Barans and Pearse Webster (SCDNR) were especially helpful in providing specimens from the Atlantic Ocean. For squid samples from the northern Gulf of Mexico, Chief Scientist Nathaniel Sanders (NMFS, Southeastern Fisheries Science Center, Pascagoula, Mississippi) allowed me to participate in the 1995 Summer Shrimp-Groundfish Survey by the R/V *Oregon* II; furthermore, his crew collected samples for me during those portions of the cruise which I could not attend. Chief Scientist Mark Grace (NMFS, Southeastern Fisheries Science Center) extended the same courtesy to me onboard the

R/V *Chapman* for the Fall 1995 Small Pelagics Survey. In addition, commercial fishermen supplied a crucial sample of *L. plei* from the Dry Tortugas-Key West area at the behest of Douglas Gregory (National Sea Grant marine extension agent, Key West, Florida). I also thank Christopher Combs (National Sea Grant marine extension agent, Brevard County, Florida) for trying to provide squid from the Atlantic coast of Florida; although the squid have yet to materialize, it has not been for lack of effort on his part.

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Along with many other people at LSU, Jason Curole, Jonathan Flowers, Adam Hrincevich, Scott Nunez, and Dr. Axayacátl Rocha-Olivares periodically provided either technical assistance or insightful conversations that helped me continue my research. Finally, I thank my family and my wife (Linda M. Heffernan) for their support over these past years.

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ABSTRACT

Based on restriction fragment length polymorphisms (RFLP) in mitochondrial DNA, the population structures of two species of *Loligo* squid were analyzed in the context of the classic Gulf of Mexico-Atlantic Ocean phylogeographic pattern. A 709-bp fragment of the cytochrome *c* oxidase (subunit I) gene was amplified by PCR from 356 *L. pealei* and 431 *L. plei*. Between latitudes 25°N and 43°N, each species had three common (> 5% frequency) haplotypes and many rare haplotypes. Sequence data from all haplotypes indicated that nucleotide divergence between the two species ranged from 13.7 to 15.0% (transition-transversion ratio of ~1.5; two inferred amino acid replacements). Within each species, RFLP analyses detected 40 to 45% of the total variability in nucleotide sites ($n = 18$ in *L. pealei*; $n = 21$ in *L. plei*). Intraspecific divergences were typically < 1%, with transition-transversion ratios of 17:4 for *L. pealei* and 21:0 for *L. plei*. Minimum-spanning networks of the sequence data showed two discrete clusters of haplotypes for *L. pealei* and no discrete clusters for *L. plei*. Both species were composed of two populations ($P < 0.02$), according to analyses of their haplotype frequencies by AMOVA (Analysis of Molecular Variance in Arlequin 1.1). *L. pealei* was divided at Florida, with one population in the northern Gulf of Mexico and another in the Atlantic Ocean; *L. plei* was divided at the Mississippi River, with one population in the northwestern Gulf of Mexico and another in the northeastern Gulf of Mexico and the Atlantic Ocean. Gene flow within each population of *L. pealei* was consistent with panmixia, while gene flow within each population of *L. plei* conformed to an isolation-by-distance model. The different phylogeographic patterns might result from the more offshore position of *L. pealei* in conjunction with the temperature and salinity tolerances of both species. In addition, the two populations of *L. plei* might represent annual recolonization from more southern populations. Finally, should fisheries develop for these species throughout the study area, these data provide support for management plans based on multiple stocks.

INTRODUCTION

Phylogeographic Context: For various marine taxa of North America, populations in the Gulf of Mexico are genetically divergent from those in the northwestern Atlantic Ocean. This phylogeographic pattern is seen in species such as the American oyster (*Crassostrea virginica*), the horseshoe crab (*Limulus polyphemus*), and the black sea bass (*Centropristis striata*). Even some birds and freshwater fishes from habitats associated with the two regions show similar population genetic structures (Avise, 1992, 1996). Given this concordance across phylogenetically divergent taxa, Avise has proposed that shared geological events (during the Pleistocene and Pliocene epochs) are largely responsible for the observed patterns.

For each species, life history characteristics appear to play a role in whether any signal of these geological events remains in the form of population genetic structure (e.g., as defined by F_{st} values). The marine species noted above are estuarine-dependent for part or all of their life cycles, and while their larvae have high dispersal potential, the adults are either sessile or have limited dispersal potential. Thus, the strong easterly currents of the Gulf Stream as it passes through the Straits of Florida (Lee et al., 1994) would tend to prevent gene flow from the Atlantic into the Gulf. Further, the Gulf Stream moves farther offshore north of Cape Canaveral, so passive gene flow from the Gulf into the Atlantic is likely to be minimal north of Florida. As a result, F_{st} values are high for such species when comparing Gulf and Atlantic populations. For estuarine-dependent fishes in which the adults are strong swimmers (e.g., red drum, *Scianops ocellatus*), the phylogeographic pattern is less pronounced, although it sometimes remains statistically significant. For these species, the lack of contiguous nursery habitat (i.e., marshes) around much of the Florida peninsula might limit genetic contact between the two regions (Gold & Richardson, 1998a). Finally, for pelagic species such as king mackerel (*Scomboromorus cavalla*) or greater amberjack (*Seriola dumerili*), F_{st} values between putative Gulf and Atlantic populations are extremely low. The low F_{st} values in conjunction

with mark-and-recapture data indicate that limited present-day gene flow occurs between the populations of both species (Gold & Richardson, 1998a,b).

Members of the neritic squid family Loliginidae (Cephalopoda: Suborder Myopsida) are also pelagic organisms, and two species of *Loligo* (*L. pealei* and *L. plei*) have distributions that encompass major parts of the Gulf of Mexico and the western Atlantic Ocean. The range of *L. pealei* (longfin squid) extends from about 46°N (Nova Scotia) to 10°N (Orinoco Delta, Venezuela), with a biomass center of distribution off the northeastern coast of the United States. In contrast, *L. plei* (arrow squid) ranges from about 35°N (Cape Hatteras) to 35°S (northern Argentina), with a center of distribution in the Caribbean Sea (Arocha & Urosa, 1991; Cohen, 1976; Roper et al., 1984; Voss et al., 1973). Compared to other species for which Gulf versus Atlantic distributions have been studied, cephalopods have short generation times (O'Dor & Webber, 1986). Statolith-based aging techniques have shown that longfin squid live about one year (Brodziak & Macy, 1996); the lifespan of arrow squid is believed to be similar. These short lifespans are thought to be responsible for episodic population expansions and collapses in cephalopods (O'Dor & Coelho, 1993), a process that could lead to population structure if the collapses create small, isolated populations. Indeed, several allozyme studies have found that other loliginids, which previously were believed to consist of panmitic populations spanning extensive oceanic ranges, are subdivided into multiple populations. In some cases, it was discovered that cryptic sibling species comprise what had been considered one species by morphology (Brierley et al., 1993; Brierley et al., 1995; Pierce et al., 1994b; Yeatman & Benzie, 1994). Nevertheless, both passive dispersal of squid paralarvae and long-distance migrations by the adults (O'Dor & Coelho, 1993) might be expected to homogenize population genetic structure in these species, leading to low F_{st} values for samples taken from the Gulf versus the Atlantic.

The reproductive habits of *Loligo* spp. theoretically should also tend to homogenize their population genetic structures. For instance, spawning by longfin squid and arrow squid occurs all along the coast in nearshore waters rather than in localized areas. Further, they are demersal spawners that typically lay eggs in large communal masses (although small groups or pairs may also lay eggs), and spawning aggregations can contain as many as hundreds of thousands of adults (Hanlon, 1998). These species will attach their egg masses to any hard substrate or even anchor them in sand (Vecchione, 1988), so there should be no lack of suitable habitat around the Florida peninsula (unlike for estuarine-dependent fishes). Reproduction in *Loligo* spp. is commonly referred to as semelparous (i.e., individuals spawn once and die immediately afterwards), but mass mortality on the spawning grounds has been observed only in *L. opalescens*. For longfin and arrow squid, it is unknown whether the two species are truly semelparous or whether they spawn intermittently over several months (Hanlon, 1998). In the laboratory, female longfin squid will mate with several males, and then lay egg capsules either immediately or over several weeks (Hanlon et al., 1997). Field studies have also detected behaviors by both sexes of longfin squid that would lead to multiple-paternity within and among egg capsules of individual females (Hanlon et al., 1997; Hanlon, 1998; Waller & Wicklund, 1968). For example, while a paired-male is displaying an agonistic posture towards another male, extra-pair copulation can occur between the paired-female and a smaller male. Finally, regardless of whether individual squid breed once or several times during their short lifespans, spawning by both species does occur throughout most of the year (Brodziak & Macy, 1996; Hanlon & Messenger, 1996; Hixon, 1980; Summers, 1983).

Despite the substantial migratory abilities of squid (O'Dor, 1988) and the breeding habits noted above, there is ample reason to expect population structure within longfin squid and arrow squid. North of Central America, the range for longfin squid extends across approximately 5,000 km of the continental shelf, while arrow squid is found adjacent to about

3,500 km of coastline. At a minimum, these ranges span four biogeographic boundaries: Cape Hatteras and Cape Canaveral in the Atlantic Ocean; the Florida peninsula between the Atlantic and the Gulf of Mexico; and, the Pensacola Bay region within the northern Gulf of Mexico. These boundaries mark where environmental and biological factors change, partitioning the marine environment into biogeographic provinces with markedly different species compositions (Briggs, 1995). For species which have ranges spanning more than one biogeographic province, Avise (1992, 1996) proposed that there should be concordance between recognized biogeographical boundaries and phylogeographic boundaries (i.e., abrupt geographic partitions of intraspecific genotypes). This hypothesis envisions that the factors creating biogeographic boundaries will also affect population genetic structure by natural selection or by impairing gene flow.

Null Hypotheses: By morphological criteria, both longfin and arrow squid are continuously distributed throughout their ranges and *Loligo* spp. are physically capable of major migrations. Thus, if these species were found to exhibit population differentiation, the expected pattern was one of gradual changes in haplotype frequencies through genetic isolation by distance (by genetic drift and natural selection). Environmental factors such as ocean currents might inhibit gene flow, increasing values for measures of population subdivision (e.g., F_{st}), but these factors are not presumed to elicit abrupt genetic breaks in a migratory pelagic species. Abrupt breaks would be anticipated only if these squids were composed of cryptic species complexes. The degree of morphological similarity between longfin squid and arrow squid is especially high within their zone of sympatry, suggesting that cryptic species might exist; further, it raises the possibility that hybridization might occur between the two species. Although hybridization had not previously been documented in cephalopods, its occurrence between these two species was supported by the discovery of six specimens that were classified as longfin squid by total protein electrophoresis, but as arrow squid by

morphometrics (Sánchez, 1995). Hence, the following null hypotheses were proposed for the present study covering the northern Gulf of Mexico and the northwestern Atlantic Ocean.

H_0-1: Neither species is composed of cryptic species within the study area.

H_0-2: One of the original classifications (electrophoretic or morphometric) of the six putative hybrids was incorrect, and the specimens were either longfin squid or arrow squid.

H_0-3: Gene flow within both species is consistent with a model of panmixia (i.e., $F_{st} = 0$ across the study area).

H_0-4: Population structure differing from panmixia is concordant with the classic Gulf of Mexico-Atlantic Ocean phylogeographic pattern that has been seen for other marine taxa in the region.

Existing Data on Population Structure: Currently, the taxonomy of Loliginidae is muddled, and the genus *Loligo* is especially problematic (Vecchione et al., 1998). In fact, morphological and genetic studies have each indicated that the genus *Loligo* is polyphyletic. Among other findings, Anderson's (1996) cladistic analysis of morphological characters for 48 loliginid species supported Brakoniecki's (1986) proposed resurrection of the name *Doryteuthis plei* for *Loligo plei*; Brierley and Thorpe (1994) suggested that *L. gahi* be removed from the genus based on substantial differentiation at the allozyme level; and, Brierley et al. (1996) used allozymes to reassign *L. edulis* and *L. chinensis* to the new genus *Photololigo*. While the higher taxonomic levels have received much attention, population level research has been less common. Prior to this study, such data were minimal for both *L. pealei* and *L. plei*.

Morphological characters are the traditional tool for identifying both species and intraspecific population structure. For instance, Cohen (1976) split *L. pealei* into two populations (Gulf versus Atlantic) based on gill length and the number of transverse sucker rows on the tentacular club. Also, based on body and beak morphometrics, Pierce et al.

(1994b) suggested that *L. forbesi* in the Azores might be a distinct stock from *L. forbesi* on the European continental shelf. However, cephalopods are composed primarily of soft tissues which leads to different measurement values among researchers and to growth patterns that are highly responsive to environmental variables (Cohen, 1976; Pierce et al., 1994a; Shaw et al., 1999). The environmental effect is evident for longfin and arrow squid because the similarity of their appearances becomes more pronounced in their zones of sympatry; specimens smaller than about 10 cm (mantle length) are especially difficult to distinguish (Sánchez, 1995). Thus, populations defined by morphology have remained suspect.

There is only one previous genetic population study of *L. pealei* in North American waters (Garthwaite et al., 1989); no such study exists for *L. plei*. For the nearly 1,000 longfin squid captured from Cape Hatteras to Cape Cod (36°N – 42°N), Garthwaite et al. found only six polymorphic allozyme loci out of the 19 examined. Further, for five loci, the variant alleles had frequencies of < 1%; only phosphoglucomutase (*Pgm*) had allele frequencies that were sufficiently variable (2.8 – 7.5% among sample units) to elucidate population structure. As analyzed, the data suggested that there were at least three populations of longfin squid north of Cape Hatteras. However, the most striking feature of this allozyme data set was that none of the 109 Virginia squid carried the variant 1.18 allele. Curiously, the frequency of that allele averaged ~2.8% in the four sample units to the north and was 5.4% to the south at Cape Hatteras (North Carolina). Evidence for population structure largely disappears if the six sample units are recombined to form three geographically distinct units (North Carolina, Virginia, and Delaware – Woods Hole and Cape Cod – Georges Bank). In any case, the low polymorphism of allozymes in longfin squid found by Garthwaite et al. (1989), as well as by researchers looking at other loliginids, demonstrates that allozyme electrophoresis is not the best method for detecting intraspecific population structure in members of the *Loligo* genus (Brierley & Thorpe, 1994; Brierley et al., 1995; Brierley et al., 1996; Yeatman & Benzie,

1994). In contrast, because two squid species are usually fixed for different alleles at polymorphic loci, allozymes are an excellent tool for testing for hybridization (Appendix A).

Current Study: DNA analyses have greater power than allozymes to detect population structure, especially for squid in which allozyme polymorphism is low. Through the use of sequence-specific primers, the polymerase chain reaction (PCR) can produce millions of copies of the desired DNA which can be analyzed for sequence variation between specimens (Dowling et al., 1996). A wide range of potential markers is available: (1) non-coding nuclear DNA (e.g., introns and microsatellites); (2) protein-coding nuclear DNA (e.g., ribosomal genes); (3) non-coding mitochondrial DNA (e.g., the control region); and, (4) protein-coding mitochondrial genes. In many evolutionary and population studies, non-coding DNA is targeted because it has fewer constraints against non-synonymous nucleotide substitutions than do protein-coding genes (Lee et al., 1995). However, intraspecific populations are unlikely to be sufficiently divergent to saturate the synonymous substitution sites with mutations (i.e., to degrade the genetic signal by multiple changes at the same sites). Given that the rate of synonymous nucleotide substitutions does not appear to differ between the unconstrained and the protein-coding regions of mitochondrial DNA (Simon et al., 1994), even protein-coding genes can be useful for elucidating population genetic structure.

When this project began, no microsatellite primers existed for either *L. pealei* or *L. plei*. Further, the location of the control region in the mitochondrial molecule is unknown for cephalopods. Thus, for use with these species, I attempted to characterize two nuclear markers (an NF-70 nuclear intron from a neuronal filament protein gene; and, ITS-1, the internal transcribed spacer region between the 18S and 28S ribosomal genes) and a mitochondrial marker (cytochrome *c* oxidase, subunit I). Although the nuclear markers appeared promising (Appendix B), only the mitochondrial marker was developed for use in this study.

The cytochrome oxidase gene was chosen for the following reasons. For cephalopods, there were published mitochondrial primer pairs for 16S rRNA and cytochrome *c* oxidase (CO-I, CO-II, and CO-III). The 16S rRNA gene was used by Bonnaud et al. (1994) to study intrafamilial taxonomic questions. The CO-II and CO-III genes were used by Bonnaud et al. (1997) to investigate cephalopod phylogeny at the order level. Similarly, the CO-I gene has been used for elucidating order and family relationships within the Subclass Coleoidea (Carlini & Graves, 1999) and intrafamilial relationships within Loliginidae (noted in Anderson, 1996) and within Sepiolidae (Nishiguchi et al., 1998). Nevertheless, CO-I has also exhibited moderate to extreme levels of intraspecific variation in other organisms (e.g., the copepod *Tigriopus californicus*; Burton, 1998); thus, this gene has demonstrated potential for use in population-level studies.

For both species, nucleotide variation in the mitochondrial CO-I gene was detected primarily by digesting the PCR products with several restriction enzymes, and population structure was determined by examining the frequencies of restriction haplotypes throughout the study area. The cryptic species hypothesis is evaluated with regard to nucleotide divergences among CO-I sequences (between and within the described species), and the hybridization hypothesis is discussed in Appendix A (Hybrid Analysis) with regard to allozyme and CO-I analyses. The two null hypotheses regarding phylogeography are evaluated by comparing the observed population genetic structures in terms of the life history characteristics and ranges of both species. Further, tests are proposed for hypotheses used to explain the phylogeographic patterns identified by the molecular data. Finally, some implications of the findings for fishery management are explored.

MATERIALS AND METHODS

Specimen Collection: The initial specimens of *L. pealei* (longfin squid) and *L. plei* (arrow squid) were provided by the Gulf Coast Research Laboratory in Ocean Springs, Mississippi. These specimens were collected from the northern Gulf of Mexico during the summer of 1993; their identities were established by Sánchez (1995) through morphometric analyses and species-specific bands on silver-stained, total-protein acrylamide gels. From 1995 to 1997, additional specimens were collected from most of the range of each species within North American waters (Figs. 1 – 3). Collectors included the U.S. National Marine Fisheries Service (Northeast and Southeast Fisheries Science Centers), the South Carolina Department of Natural Resources, and commercial fishermen (Dry Tortugas pink shrimp fishery, Florida).

Specimens collected after 1993 were frozen at -20°C on board ship. In the laboratory, mantle tissue samples (100 – 300 mg) were taken from 356 longfin squid and from 431 arrow squid (including the 1993 specimens) and stored at -70°C. Longfin specimens ranged in size (mantle length) from 35 to 460 mm ($\bar{x}$ = 153 mm ± 63 SD) while arrow squid specimens ranged from 15 to 275 mm ($\bar{x}$ = 89 mm ± 47 SD). To minimize contamination of tissue samples by other squid tissue, the excision site was rinsed with saline and clean mantle tissue was exposed by scraping off the integument with an autoclaved razor blade. Each sampled squid was then individually bagged, labeled (including the latitude and longitude of capture), and stored at -20°C for possible morphological analysis (Appendix C, Tables C-1, 2, and 3).

PCR (Polymerase Chain Reaction): Total genomic DNA was extracted from each specimen (10 – 20 mg mantle tissue) with a phenol-chloroform procedure (Appendix D; modified from Hillis et al., 1996), ethanol precipitated, dried, and then resuspended in 50 µl of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0). Resuspended DNA was stored at -80°C. HPLC-grade water (Mallinckrodt Baker Inc., Paris, KY) and filter-pipette tips (USA Scientific Plastics, Ocala, FL) were used for DNA extraction and amplification.

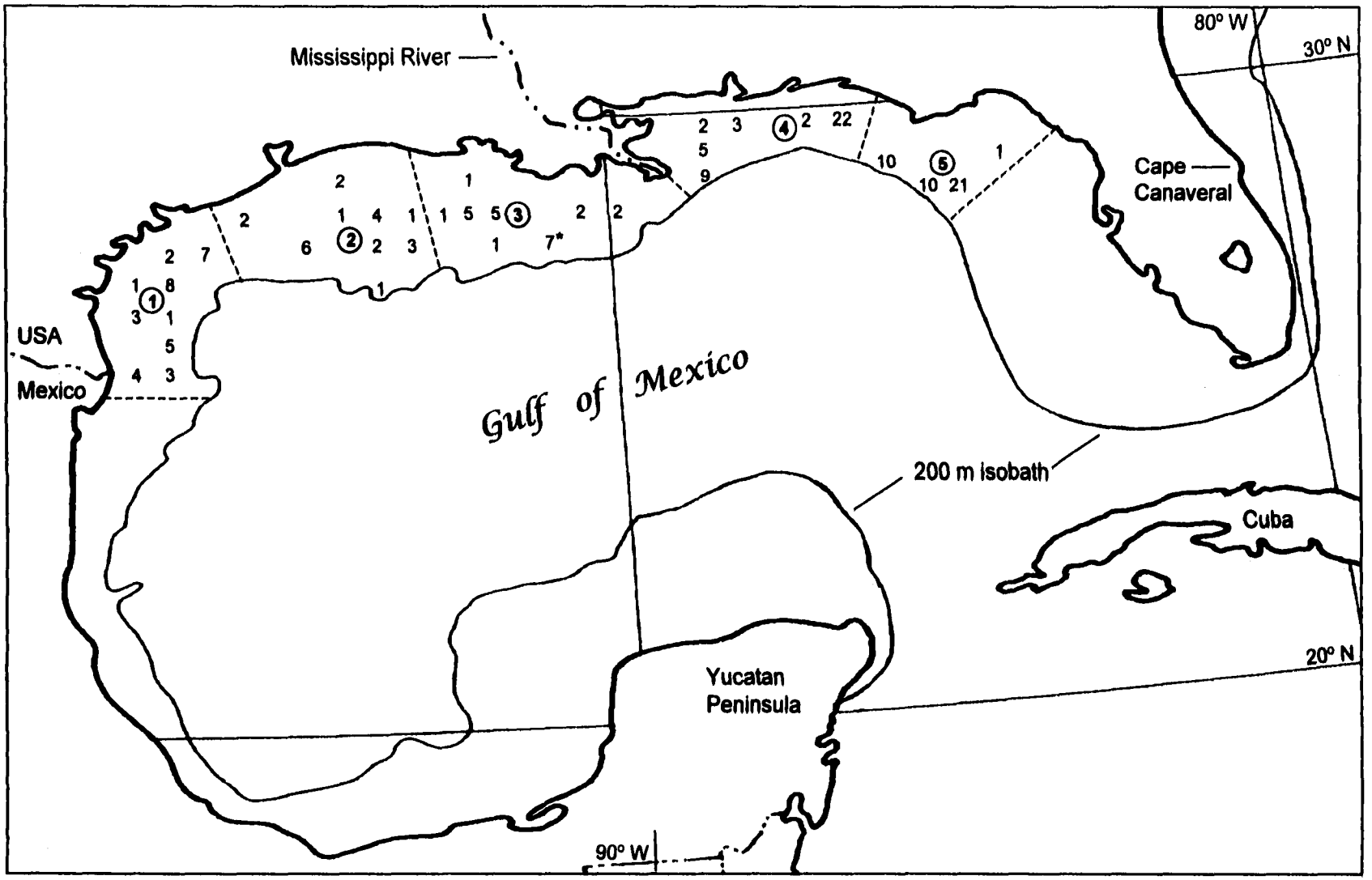

Fig. 1. *Loligo pealei* (longfin squid): Sample units ① to ⑤ (separated by dashed lines), depicting the number of squid analyzed from combined Gulf of Mexico trawl stations. All positions were rounded to the nearest 0.5 degrees latitude and longitude, and the unit number marks the approximate multivariate mean of trawl stations within each sample unit. Locations of seven squid (7*) within unit ③ are unknown.

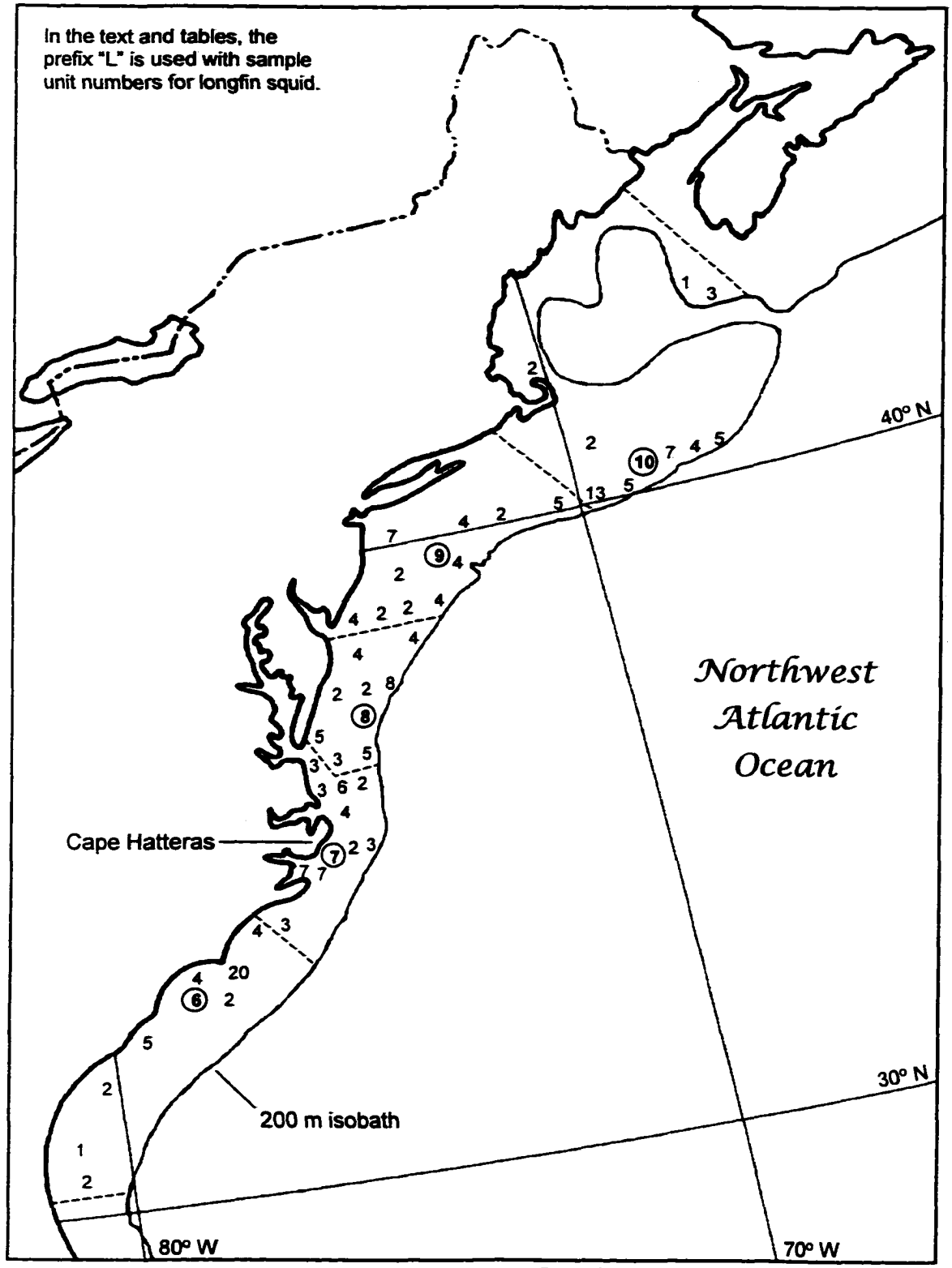

Fig. 2. *Loligo pealei* (longfin squid): Sample units ⑥ to ⑩ (separated by dashed lines), depicting the number of squid analyzed from combined Atlantic Ocean trawl stations. All positions were rounded to the nearest 0.5 degrees latitude and longitude, and the unit number marks the approximate multivariate mean of trawl stations within each sample unit.

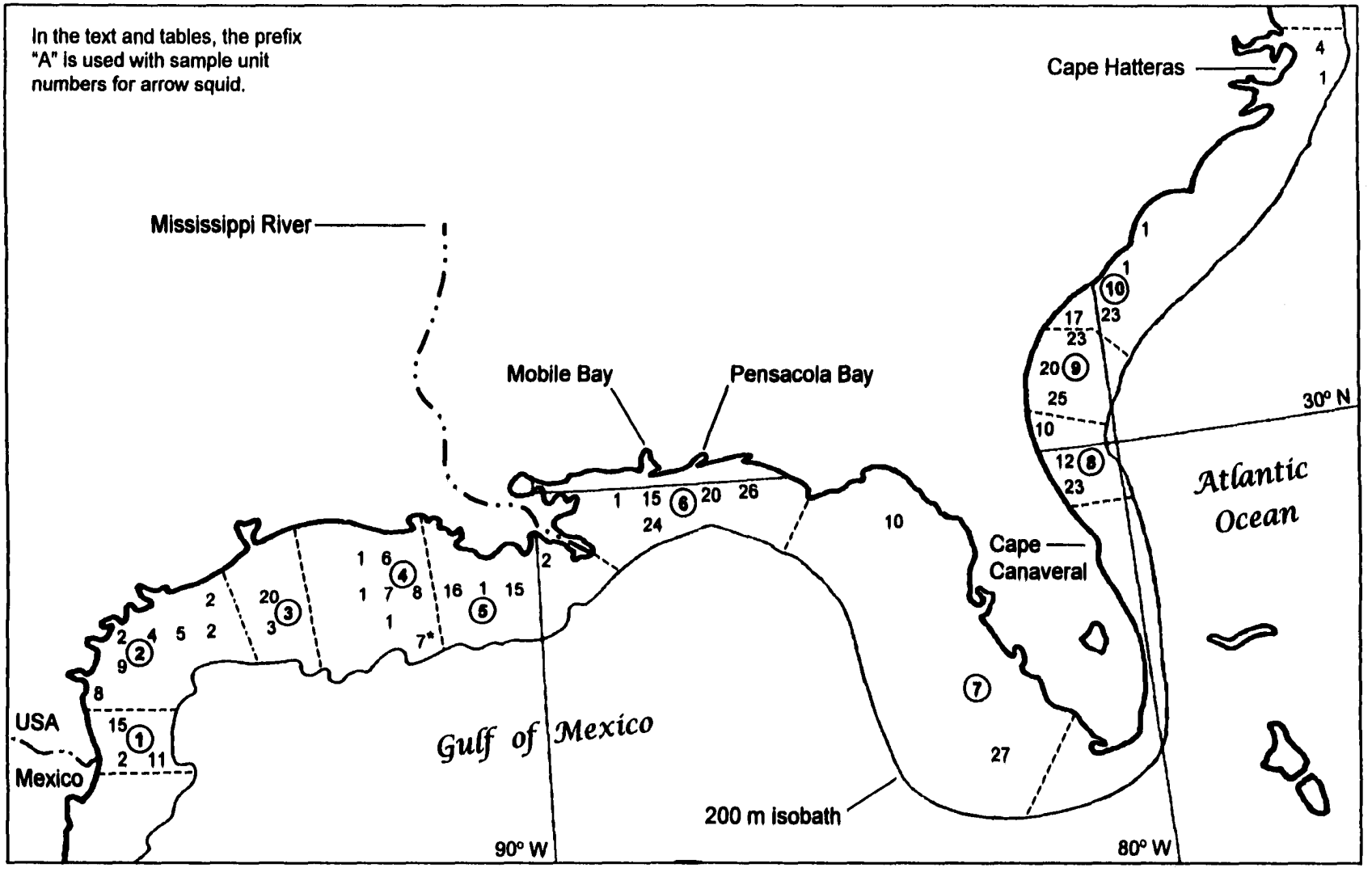

Fig. 3. *Loligo plei* (arrow squid): Sample units ① to ⑩ (separated by dashed lines), depicting the number of squid analyzed from Gulf and Atlantic trawl stations. All positions were combined to nearest 0.5 degrees latitude and longitude, and the unit number marks the approximate multivariate mean of trawl stations within each sample unit. Locations of seven squid (7*) within unit ④ (or ⑤) are unknown (see Table C-3).

The mitochondrial DNA (mtDNA) marker was a 709-bp fragment of the gene cytochrome *c* oxidase, subunit I (CO-I). Amplifications used the universal primers of Folmer et al. (1994): [H-COI] 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'; [L-COI] 5'-GGTCA ACAAATCATAAAGATATTGG-3'. PCR was done on a Perkin-Elmer DNA Thermocycler, and each 50 µl reaction was capped with 25 µl of Chill-out 14 wax (MJ Research, Watertown, MA) to prevent evaporation during cycling. Final reagent concentrations were 180 µM each dNTP, 1X rec-*Tbr* buffer (Amresco, Solon, OH), 1.9 mM $MgCl_2$, 15 pmol of each primer, and 1.0 µl of DNA extract. The "hot start" consisted of holding the reactions at 95°C for 5 min, reducing the block temperature to 90°C, and then adding 5 µl of diluted (1 unit/5 µl) rec-*Tbr* polymerase to each tube. Following the "hot-start", there were 43 cycles of 95°C (30 s); 50°C (30 s); and 72°C (30 s). To make it easier to dispense DNA for the restriction digests, all completed reactions were diluted with water to a final volume of 100 µl. Reactions were repeated, using new DNA extractions, if 5 µl of the diluted PCR products failed to produce bright, discrete bands in 2% agarose gels (stained with ethidium bromide).

Restriction Digests: RFLP (Restriction Fragment Length Polymorphism) analyses were the primary means of detecting nucleotide variation in the PCR products. Restriction digests were done in 25 µl total volumes according to the manufacturers' protocols. Depending on their expected sizes, fragments from a digest were separated in either 2% or 3% Agarose 3:1® gels (Amresco, Solon, OH) submerged in 0.5X TBE buffer (0.22 M Tris-HCl, 0.22 M boric acid, 7 mM EDTA, pH 8.0). DNA fragments were stained by adding ethidium bromide to both the molten agar and the running buffer. Prior to sequencing, fragment sizes were estimated by comparison to a Hi-Lo DNA marker (Minnesota Molecular, Minneapolis, MN) in 8% non-denaturing, polyacrylamide gels. Polaroid 667 film (ISO 3000) was used to document all gel results.

The RFLP analysis was initiated with the identified specimens from 1993. First, several PCR products from both species were digested with 23 separate restriction enzymes: *Ase*I, *Ava*II, *Bcl*I, *Bst*NI, *Cfo*I, *Dde*I, *Dpn*II, *Dra*I, *Eco*RI, *Hae*III, *Hin*dIII, *Hin*fI, *Hsp*92II, *Mse*I, *Msp*I, *Nsi*I, *Rsa*I, *Scr*FI, *Sma*I, *Ssp*I, *Stu*I, *Taq*I, and *Xba*I (primarily from New England Biolabs, Beverly, MA). Endonucleases that cut PCR products from at least one species were tested for intraspecific polymorphisms (in ≥ 40 squid). Finally, based on the ability of each enzyme to distinguish the two species and to generate unambiguous, intraspecific RFLP's in agarose gels, a subset of enzymes was chosen for each species: *Bst*NI, *Hae*III, *Hin*fI, and *Msp*I for longfin squid; and *Ase*I, *Hsp*92II, and *Msp*I for arrow squid. The data from the digestion profiles of all enzymes were combined such that each specimen was assigned to a PCR-RFLP haplotype. *Msp*I provided a single enzyme test for species verification because it always cut the PCR products of both species, and the interspecific RFLP patterns for *Msp*I were distinct.

DNA Sequencing: Representatives of each haplotype were sequenced with both primers (n = 23 squid for *L. pealei*; n = 22 squid for *L. plei*). For haplotypes represented by more than one specimen, PCR products from at least two squid were sequenced, and those specimens were selected from the most geographically distant sample sites. PCR products were purified with QIAquick columns (QIAGEN, Valencia, CA) and resuspended in 30 µl of elution buffer (10 mM Tris-HCl, pH 8.5). Sequencing reactions were performed with the ABI-Prism Dye-Terminator kit from Applied BioSystems (Perkin-Elmer, Norwalk, CT). Each reaction contained 2.0 µl Prism mix, 5.5 µl water, 3.0 µl of either the H-COI or L-COI primer (1 µM), 1.5 µl DNA, and 16 µl Chill-out wax. After a 5 min hot-start (90°C), reactions were subjected to 25 cycles of 96°C (30 s), 50°C (30 s) and 60°C (4 min). Labeled extension products were separated on acrylamide gels and analyzed with an automated DNA sequencer (Applied BioSystems model 373A) at the Museum of Natural Sciences, Louisiana State University.

For each specimen, the complementary CO-I sequences were compared in Sequencher 4.0 (Gene Codes Corp., Ann Arbor, MI) to ensure accurate nucleotide assignments. Sequences were trimmed to 658 bp (by excision of primer locations) and aligned in ClustalW v. 1.7 (Thompson et al., 1994). Transition-transversion ratios and percent sequence divergences were calculated in MEGA (Sudhir et al., 1993). Intraspecific haplotype relationships were determined by analyzing the CO-I sequences by maximum-likelihood methods in Paup* 4.0b2 (Sinauer Associates Inc., Sunderland, MA). Further, for each species, distance matrices (Felsenstein, 1984) using all sequences were generated by Paup* and processed in MINSPNET (Excoffier, 1993; http://anthropologie.unige.ch/~laurent) to calculate minimum-spanning networks, which were graphed by hand. The above results were used to (1) confirm restriction sites within each PCR-RFLP haplotype, (2) identify homoplasic haplotypes, (3) generate accurate genetic distances among haplotypes, and (4) aid in determining whether a haplotype might represent a cryptic species.

Genetic Structure Analysis: The federal and state surveys used a randomized block design to determine the locations of trawl stations along the coast. Further, because schools of squid might be genetically homogeneous, specimens were selected from a wide array of the available trawl stations. Within each species, specimens were grouped by capture locations to the nearest 0.5 degrees of latitude and longitude; they were then clustered into seven sample units (of roughly-equal sizes) within the Gulf of Mexico and seven units within the Atlantic Ocean. Inspection of this haplotype frequency data revealed the possibility of two populations for longfin squid (Gulf of Mexico versus Atlantic Ocean) and two populations for arrow squid (west versus east of the Mississippi River).

For each species, the haplotype frequencies across the sampling range were analyzed by bar graphs and by the AMOVA (Analysis of Molecular Variance) package in Arlequin 1.1 (Schneider et al., 1997). Statistical significance in AMOVA is calculated by a nonparametric

permutation test, which randomly recombines the data at each level of the analysis (versus true permutation). AMOVA simulations were run to evaluate the ability of the program to recognize population structure when only two major populations (i.e., groups) were postulated. These simulations showed that the statistical power of AMOVA is limited unless the two groups contain many sample units. Otherwise, despite the stated "thousands" of permutations selected for the analysis, only a few non-repetitive combinations of sample units exist. In that case, even for well-differentiated populations, the haplotype frequency variance for the "Among Groups" level might be declared non-significant (especially when haplotypes are evolutionarily equidistant). Therefore, to achieve uncorrected significance values of $P < 0.01$ in AMOVA, I needed at least 100 possible, non-repetitive combinations of sample units across regions. For each species, corrected table-wide significance values were subsequently calculated through a sequential Bonferroni procedure (Rice, 1989).

Accordingly, each species was redivided into five larger sample units per putative population [$(10! \div (5! * 5!)) \div 2 = 126$ unique combinations]. The divisions were based primarily on maintaining similar sample sizes within units and partially on the presence of major geographic features (e.g., large rivers, bays, and undersea canyons). In the text and tables, the sample unit prefix "L" designates units for longfin squid and the prefix "A" designates units for arrow squid; the prefixes are not used in the figures.

Compared to other sample units for arrow squid, about twice as many specimens were analyzed from the vicinity of Pensacola Bay. Additional samples of arrow squid (the more inshore species) were taken because the western panhandle of Florida appeared to separate genetically distinct groups of the intertidal bivalve *Rangia cuneata* in the Gulf of Mexico (Foltz et al., 1995). In addition, this area is a contact zone between distinct populations of freshwater aquatic species as well as terrestrial fauna (Avise, 1992, 1996). Thus, prior to the final AMOVA analysis, sample unit A-6 was split into one subunit south of Mobile Bay and

one subunit south of Pensacola Bay to test for a phylogeographic break in that area for arrow squid (Fig. 3). Haplotype frequencies were tested for homogeneity across the subunits by a log likelihood *G*-test (Sokal & Rohlf, 1995), and corrected significance values were subsequently calculated through a sequential Bonferroni procedure (Rice, 1989).

Patterns of gene flow within each species were illustrated through neighbor-joining trees of the coancestry coefficients for sample units; the coefficient matrices were calculated in AMOVA based on all of the original haplotypes and the trees were created in MEGA. For all other phylogeographic analyses (bar graph and AMOVA), specimens were reclassified into fewer composite haplotypes that were phylogenetically-defensible (see Results). The level of reduction necessary for the AMOVA analyses obscured information visually present in the haplotype distributions. Thus, for the bar graphs, less common haplotypes were recombined into composite haplotypes different from those used in the AMOVA analyses. In AMOVA, haplotypes within each species were treated as equidistant (i.e., no distance matrices were used). As a result, fixation indices are reported as standard F_{st} values rather than as AMOVA Φ statistics (which are partly based on the relationships among haplotypes). Following the definitions of Weir and Cockerham (1984) and Excoffier et al. (1992), F_{st} is the correlation of random haplotypes within populations relative to random pairs of haplotypes drawn from the whole species (i.e., a measure of "coancestry"); F_{is} is the correlation of the molecular diversity of random haplotypes within sample units (of a population) relative to that of random pairs of haplotypes drawn from the whole population; and, F_{it} is the correlation of random haplotypes within sample units relative to random pairs of haplotypes drawn from the whole species.

RESULTS

The CO-I mtDNA amplifications were robust from specimens collected after 1993, and all restriction enzyme digests produced fragments that summed to the size of the PCR product (Figs. 4 and 5). The RFLP analyses revealed three common (> 5% frequency) haplotypes for each species, along with many rare haplotypes (Table 1). For both species, the common haplotypes were rarely absent from sample units. However, for longfin squid, about 90% of Haplotype C and 100% of Haplotype E were found in the Atlantic Ocean while nearly 80% of Haplotype B was found in the Gulf of Mexico (Table 2). For arrow squid, the common haplotypes were found primarily either west or east of the Mississippi River (Table 3).

Sequence Comparisons: CO-I sequences were obtained from 23 *L. pealei* and 22 *L. plei*. For each PCR-RFLP haplotype, comparisons of the complementary sequences rendered unambiguous nucleotide data (658 bp, excluding primer positions). Within the consensus sequences of the common haplotypes (Fig. 6), there were 102 positions at which the two species potentially had different nucleotides (85 invariant differences; 2 variable differences; and 15 haplotype or sequence-specific differences). Two interspecific differences at first codon positions resulted in amino acid replacements (leucine for methionine; phenylalanine for valine); all other differences were silent substitutions in the first or third codon positions. Nucleotide divergence between longfin squid and arrow squid ranged from 13.7 to 15.0% and the interspecific transition-transversion ratio ranged from 1.49 to 1.72.

For longfin squid, all nucleotide differences within haplotypes were silent substitutions in the third codon position (Table 4). Considering all haplotypes, there were 17 transitions and 4 transversions (transition-transversion ratio = 4.25) at the 18 variable sites; the RFLP analysis detected nearly 45% of the variable sites. The minimum-spanning network documented two clades that centered on the numerically-dominant Haplotypes A or B; based on Crandall and Templeton (1993), Haplotypes A and B are the best candidates for the most recent common

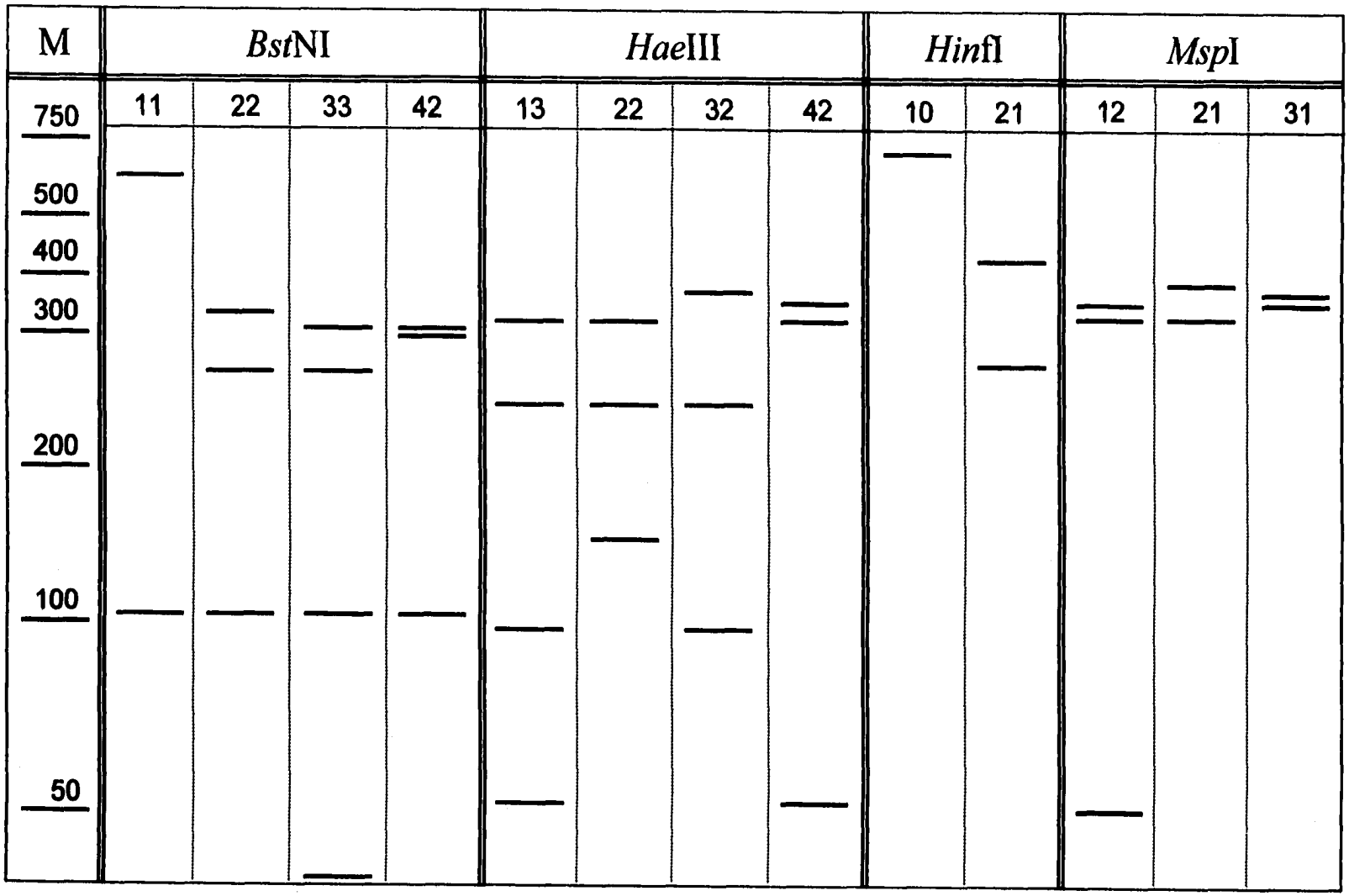

Fig. 4. *Loligo pealei* (longfin squid): Restriction digest patterns for a 709-bp fragment of the mtDNA gene cytochrome *c* oxidase, (subunit I). Lane M represents the DNA reference ladder (bp); numerals in other lanes represent the restriction digest codes used in Table 1-A for the summary of the RFLP-mtDNA haplotypes (1^{st} digit is the pattern number; 2^{nd} digit is the number of restriction sites). Bands smaller than 75 bp were not always visible on agarose gels.

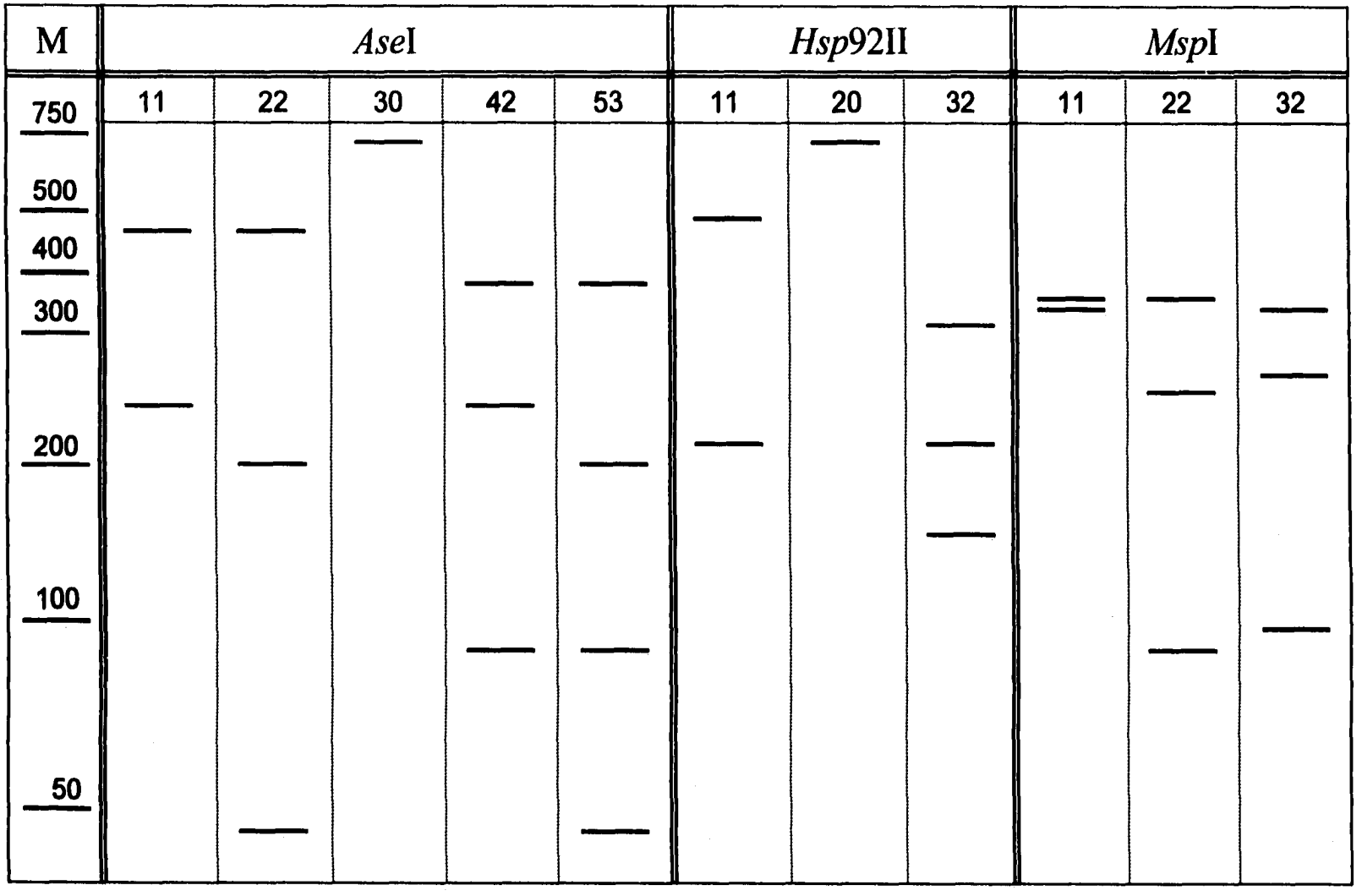

Fig. 5. *Loligo plei* (arrow squid): Restriction digest patterns for a 709-bp fragment of the mtDNA gene cytochrome *c* oxidase, (subunit I). Lane M represents the DNA reference ladder (bp); numerals in other lanes represent the restriction digest codes used in Table 1-B for the summary of the RFLP-mtDNA haplotypes (1^{st} digit is the pattern number; 2^{nd} digit is the number of restriction sites). Bands smaller than 75 bp were not always visible on agarose gels.

Table 1. PCR-RFLP haplotypes for two species of *Loligo* squid, based on separate restriction digests of a 709-bp fragment of the mtDNA CO-I gene. See Figures 4 and 5 for explanation of the binary codes for restriction digests and actual gel patterns. The recognition sequence for each restriction enzyme is shown in parentheses (IUB-IUPAC codes).

A. *Loligo pealei* (longfin squid).

Haplotypes			Restriction Enzymes			
Letter	Observed	Frequency	*Bst*NI (ccwgg)	*Hae*III (ggcc)	*Hin*fI (gantc)	*Msp*I (ccgg)
A	249	69.9 %	11	13	10	12
B	50	14.0 %	11	13	21	12
C	22	6.2 %	11	22	10	21
D	9	2.5 %	22	13	10	12
E	9	2.5 %	33	13	10	12
F	7	2.0 %	11	22	10	12
G	2	0.6 %	11	32	10	12
H	3	0.8 %	11	22	21	12
I	1	0.3 %	11	42	21	12
J	1	0.3 %	11	32	21	12
K	1	0.3 %	22	13	21	12
L	1	0.3 %	42	13	10	12
M	1	0.3 %	11	13	10	31
Total	356	100.0 %				

B. *Loligo plei* (arrow squid).

Haplotypes			Restriction Enzymes		
Letter	Observed	Frequency	*Ase*I (attaat)	*Hsp*92II (catg)	*Msp*I (ccgg)
A	232	53.9 %	11	11	11
B	92	21.3 %	11	20	11
C	95	22.0 %	22	11	11
D	2	0.5 %	30	11	11
E	1	0.2 %	11	32	11
F	2	0.5 %	11	11	22
G	1	0.2 %	42	20	11
H	2	0.5 %	22	20	11
I	2	0.5 %	42	11	11
J	1	0.2 %	22	11	32
K	1	0.2 %	53	11	11
Total	431	100.0 %			

Table 2. *Loligo pealei* (longfin squid [$n = 356$]): Absolute frequencies of the PCR-RFLP haplotypes (mtDNA CO-I), segregated by populations and sample units.

Sample Units[1]		A	B	C	D	E	F	G	H	I	J	K	L	M	Sum
L-1	(West Texas)	27	5	0	2	0	0	0	0	0	0	0	0	0	34
L-2	(East Texas)	13	6	0	1	0	0	1	0	1	0	0	0	0	22
L-3	(Louisiana)	16	5	1	1	0	1	0	0	0	0	0	0	0	24
L-4	(West Florida)	28	11	1	1	0	0	0	1	0	0	0	0	1	43
L-5	(Central Florida)	27	12	0	1	0	1	0	0	0	1	0	0	0	42
Total		111	39	2	6	0	2	1	1	1	1	0	0	1	165
Sample Units[2]		A	B	C	D	E	F	G	H	I	J	K	L	M	Sum
L-6	(South Carolina)	28	4	6	0	1	0	1	0	0	0	0	0	0	40
L-7	(North Carolina)	33	1	1	0	3	2	0	0	0	0	0	0	0	40
L-8	(Virginia)	27	3	1	0	1	1	0	0	0	0	0	0	0	33
L-9	(New Jersey)	25	2	3	1	2	1	0	2	0	0	0	0	0	36
L-10	(Massachusetts)	25	1	9	2	2	1	0	0	0	0	1	1	0	42
Total		138	11	20	3	9	5	1	2	0	0	1	1	0	191

[1] Gulf of Mexico population; [2] Atlantic Ocean population.

Table 3. *Loligo plei* (arrow squid [$n = 431$]): Absolute frequencies of the PCR-RFLP haplotypes (mtDNA CO-I), segregated by populations and sample units.

Sample Units[1]		A	B	C	D	E	F	G	H	I	J	K	Sum
A-1	(West Texas)	8	19	0	0	0	0	1	0	0	0	0	28
A-2	(Central Texas)	9	19	3	0	0	1	0	0	0	0	0	32
A-3	(East Texas)	6	12	4	0	0	0	0	1	0	0	0	23
A-4	(West Louisiana)	9	16	6	0	0	0	0	0	0	0	0	31
A-5	(East Louisiana)	11	14	9	0	0	0	0	0	0	0	0	34
Total		43	80	22	0	0	1	1	1	0	0	0	148
Sample Units[2]		A	B	C	D	E	F	G	H	I	J	K	Sum
A-6	(West Florida)	49	9	26	0	0	0	0	0	1	0	1	86
A-7	(Key West, Florida)	25	0	10	1	1	0	0	0	0	0	0	37
A-8	(North Florida)	35	0	9	0	0	0	0	0	0	1	0	45
A-9	(Georgia)	47	2	16	0	0	1	0	1	1	0	0	68
A-10	(South Carolina)	33	1	12	1	0	0	0	0	0	0	0	47
Total		189	12	73	2	1	1	0	1	2	1	1	283

[1] Population west of the Mississippi River (northwestern Gulf of Mexico); [2] Population east of the river, including the Atlantic Ocean.

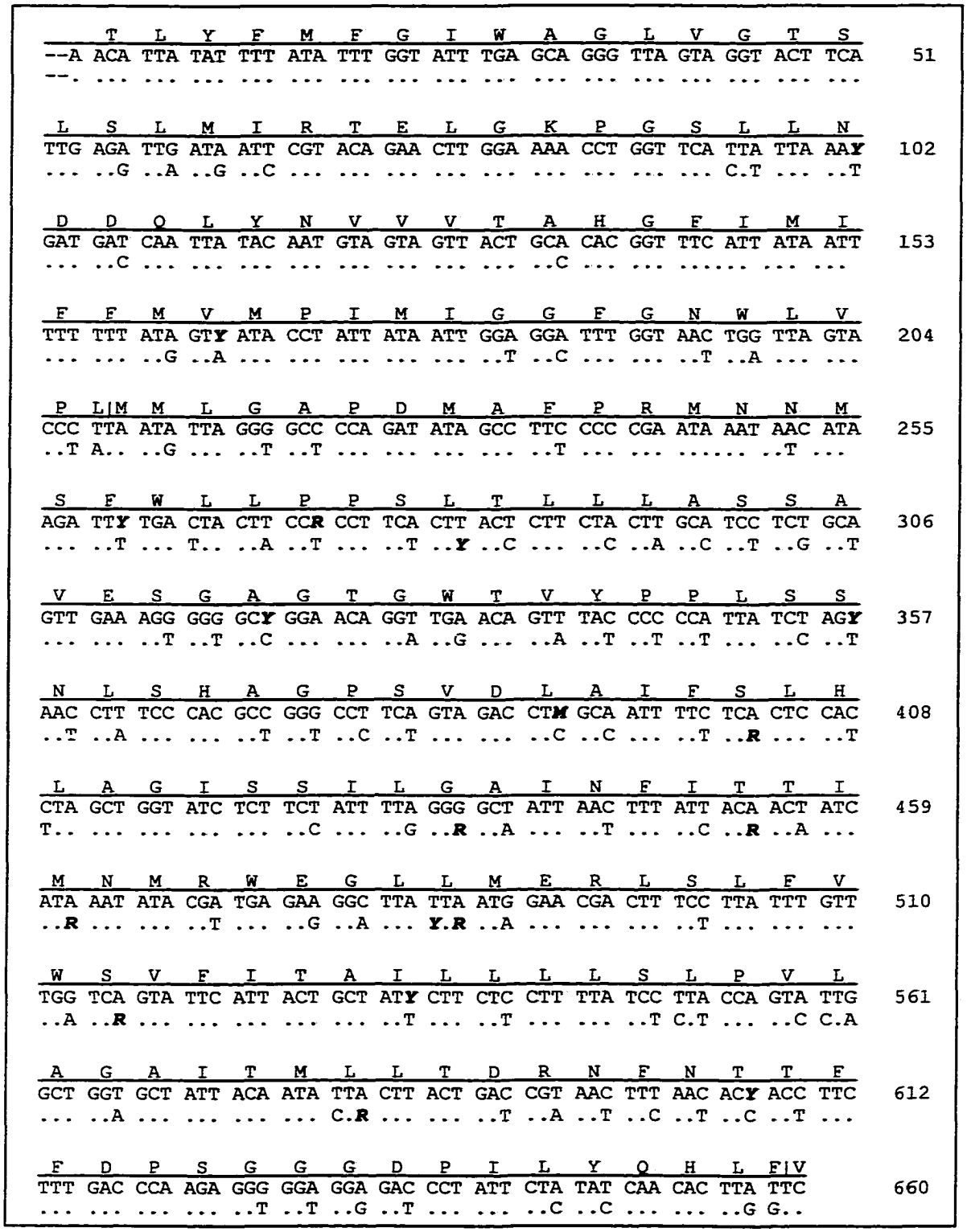

Fig. 6. Nucleotide sequences (*Loligo pealei*, top; *L. plei*, bottom) and inferred amino acid sequences for the mtDNA CO-I gene. Dots represent identity with *L. pealei*; intraspecific nucleotide variations (IUB-IUPAC codes) are in a bold, italicized font (for haplotypes that occurred at > 5% frequency). Amino acid replacements (two) for *L. pealei* are shown first.

Table 4. Variable nucleotide sites within the mtDNA sequence (cytochrome *c* oxidase, subunit I; Figure 6) of two squid species. Sites detected by RFLP analyses are shown in a bold, italicized font. "Periods" denote identity with the first sequence, and the subscripts identify multiple representatives within an RFLP haplotype (squid from which DNA was sequenced are identified in Table C-5).

Haplotype		Nucleotide Position (*Loligo pealei*)																	
		1	1	**2**	**2**	2	**3**	**3**	3	**3**	**3**	**3**	**4**	5	5	5	5	6	
		4	0	6	**1**	**6**	7	**1**	**2**	5	**7**	**7**	**9**	**1**	2	3	4	6	0
		8	2	5	**9**	**1**	3	**8**	**1**	7	**2**	**5**	**0**	**1**	5	4	9	7	6
●	A_1	T	C	T	**G**	**T**	G	**G**	**C**	T	**C**	**G**	**A**	**A**	T	C	C	T	T
	A_2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
●	A_3	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
●	B_1	.	T	.	.	**C**	A	.	.	.	.	.	**C**	.	.	T	.	.	C
	B_2	.	T	.	.	**C**	A	.	.	C	.	.	**C**	.	.	T	.	.	C
●	C_1	.	.	.	.	.	.	.	**T**	.	.	.	.	.	.	.	.	.	.
	C_2	.	.	.	.	.	.	.	**T**	.	.	.	.	.	.	.	.	.	.
	C_3	.	.	.	.	.	.	.	**T**	.	.	.	.	.	.	.	.	.	.
●	D_1	.	.	.	.	.	.	.	.	.	.	.	.	**G**	.	.	.	.	.
	D_2	.	.	.	.	.	.	.	.	.	.	.	.	**G**	.	.	.	.	.
●	E	.	.	.	.	.	T	.	.	.	.	.	**G**	**G**	.	.	.	.	.
●	F_1	.	.	.	.	.	.	**A**	.	.	.	.	.	.	.	.	.	.	.
	F_2	.	.	.	.	.	.	**A**	.	.	.	.	.	.	.	.	.	.	.
	G_1	.	.	.	.	.	.	.	.	.	.	**A**	.	.	.	.	.	.	.
●	G_2	.	.	.	.	.	.	.	.	.	.	**T**	.	.	.	.	G	.	.
●	H_1	.	T	.	.	**C**	A	**A**	.	.	.	.	**C**	.	.	T	.	.	C
●	H_2	C	T	.	.	**C**	A	**A**	.	.	.	.	**C**	.	.	T	.	.	C
	H_3	.	T	.	.	**C**	A	**A**	.	.	.	.	**C**	.	.	T	.	C	C
	I	.	T	.	**A**	**C**	A	.	.	.	.	.	**C**	.	C	T	.	.	.
	J	.	T	.	.	**C**	A	.	.	.	.	**A**	**C**	.	.	T	.	.	C
●	K	.	T	.	.	**C**	A	.	.	.	.	.	**C**	**G**	.	T	.	.	.
●	L	.	.	.	.	.	.	.	.	.	.	.	**G**	.	.	.	.	.	.
	M	.	.	.	.	.	.	.	.	.	**T**	.	.	.	.	.	.	.	.

Haplotype		Nucleotide Position (*Loligo plei*)																		
			1	**2**	2	2	3	4	**4**	4	4	**4**	4	**4**	**4**	4	4	**4**	5	5	6
		5	7	**3**	**4**	6	8	6	0	**1**	3	3	**4**	5	**5**	**6**	7	8	**8**	1	8	0
		7	2	**8**	**3**	4	2	1	2	**4**	2	5	**4**	3	**9**	**2**	7	4	**6**	6	2	6
	A_1	**G**	A	**C**	**A**	A	T	C	A	**T**	G	A	**T**	G	**C**	**G**	G	T	**G**	G	G	C
■	A_2	.	.	.	.	.	C	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.
■	B_1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**A**	.	.	.	.	.	.
■	B_2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**A**	.	.	.	A	.	.
■	B_3	.	.	.	.	.	.	.	.	.	.	.	.	A	.	**A**	.	C	.	.	.	.
■	B_4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**A**	.	.	.	.	A	.
	B_5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**A**	.	.	.	.	A	.
■	C_1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**A**	.	.	.
■	C_2	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	**A**	.	.	.
	C_3	.	.	.	.	.	.	.	.	.	.	G	.	A	.	.	.	.	**A**	.	.	.
■	D_1	.	.	.	.	.	.	.	.	.	.	.	**C**	.	.	.	.	.	.	.	.	.
■	D_2	.	G	.	.	.	.	.	.	.	A	.	**C**	.	.	.	.	.	.	.	.	T
■	E	.	.	**T**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	F_1	.	.	.	**G**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
■	F_2	.	.	.	**G**	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	G	**A**	.	.	.	.	.	.	.	.	.	.	.	.	.	**A**	.	.	.	.	A	.
	H_1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	**A**	.	.	**A**	.	A	.
■	H_2	.	.	.	.	.	.	.	.	.	.	.	.	A	**T**	.	.	.	**A**	.	.	.
■	I_1	**A**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
■	I_2	**A**	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.
■	J	.	.	.	.	G	.	.	**C**	.	G	.	.	.	.	A	.	**A**	.	.	.	.
■	K	**A**	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	**A**	.	.	.

● Squid captured in the Atlantic (versus Gulf of Mexico).

■ Squid captured east (versus west) of the Mississippi River.

ancestor within each clade because they are both geographically widespread and show the most connections to other haplotypes (Fig. 7). Nucleotide divergence between clades was about 1%; within each clade, most haplotypes could be transformed into their nearest neighbor by a single transition (~0.15% sequence divergence). It was equally parsimonious to infer that the Atlantic Haplotype G was most closely related either to Haplotype A or to the Gulf Haplotype G.

For arrow squid, all nucleotide differences within haplotypes were silent substitutions in the first or third codon positions (Table 4). Considering all haplotypes, there were 21 transitions and no transversions at the 21 variable sites (transition-transversion ratio = 21:0, or ∞). The RFLP analysis detected nearly 40% of the variable sites; despite being within an *Ase* I site, the transition at position 484 of Sequence B_3 was not detectable because the guanine at position 486 had already eliminated the restriction site. Nucleotide divergence between haplotypes was generally less than about 0.5%. The minimum-spanning network suggested that Haplotypes A, B, and C form three clades, which are very closely related (Fig. 8). For Haplotype B, the two Gulf of Mexico representatives had identical sequences, while all three specimens found in the Atlantic Ocean had unique sequences.

For both species, most haplotypes were found in the Atlantic Ocean as well as in the Gulf of Mexico, and representative samples were sequenced from both areas. Direct sequencing confirmed the restriction sites (Fig. 9), which previously had been inferred from digest patterns. In most cases, sequence determination of replicate individuals within each PCR-RFLP haplotype detected at least one nucleotide difference (Table 4). Several RFLP haplotypes contained restriction site homoplasies (i.e., convergence of DNA lineages on the absence of a specific restriction site, either through site loss in those lineages or site gain in all other lineages). In longfin squid, Haplotypes E and L contained a *Bst*NI restriction site (CCWGG) at position 388; the other haplotypes lacked the site due to the presence of two different nucleotides at the same position (CCTAG for Haplotypes A, C, D, F, G, M; CCTCG

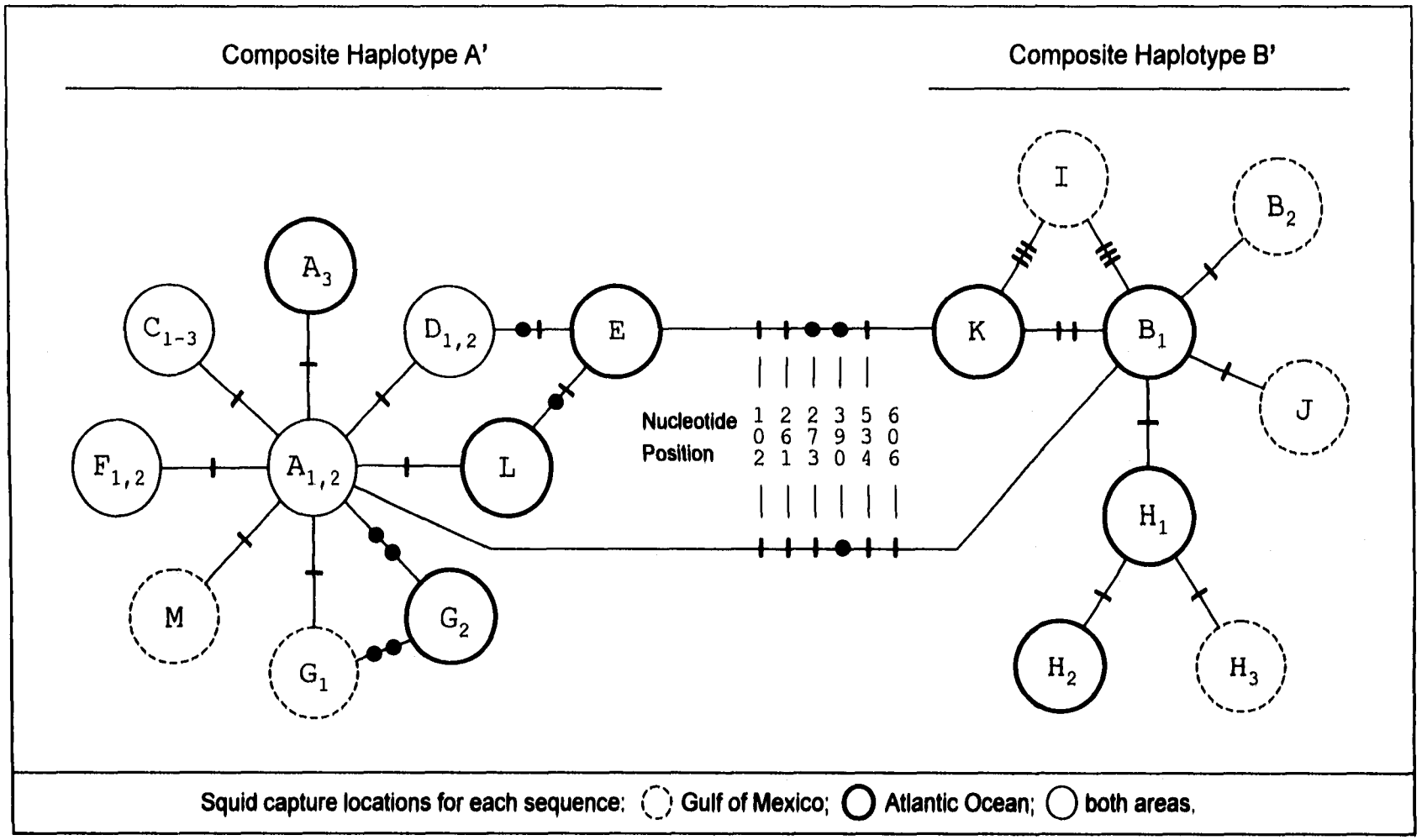

Fig. 7. *Loligo pealei* (longfin squid): Minimum spanning network of CO-I nucleotide sequences for the mtDNA PCR-RFLP haplotypes (A-M, as defined in Table 1-A). Each circle represents one or more DNA sequences as defined in Table 4, with nearest neighbors separated by transitions (cross-bars) and transversions (dots). Composite haplotypes A' and B' were used in the AMOVA analyses. Haplotype G might be composed of two mtDNA lineages that have converged on a PCR-RFLP haplotype (i.e., homoplasic lineages).

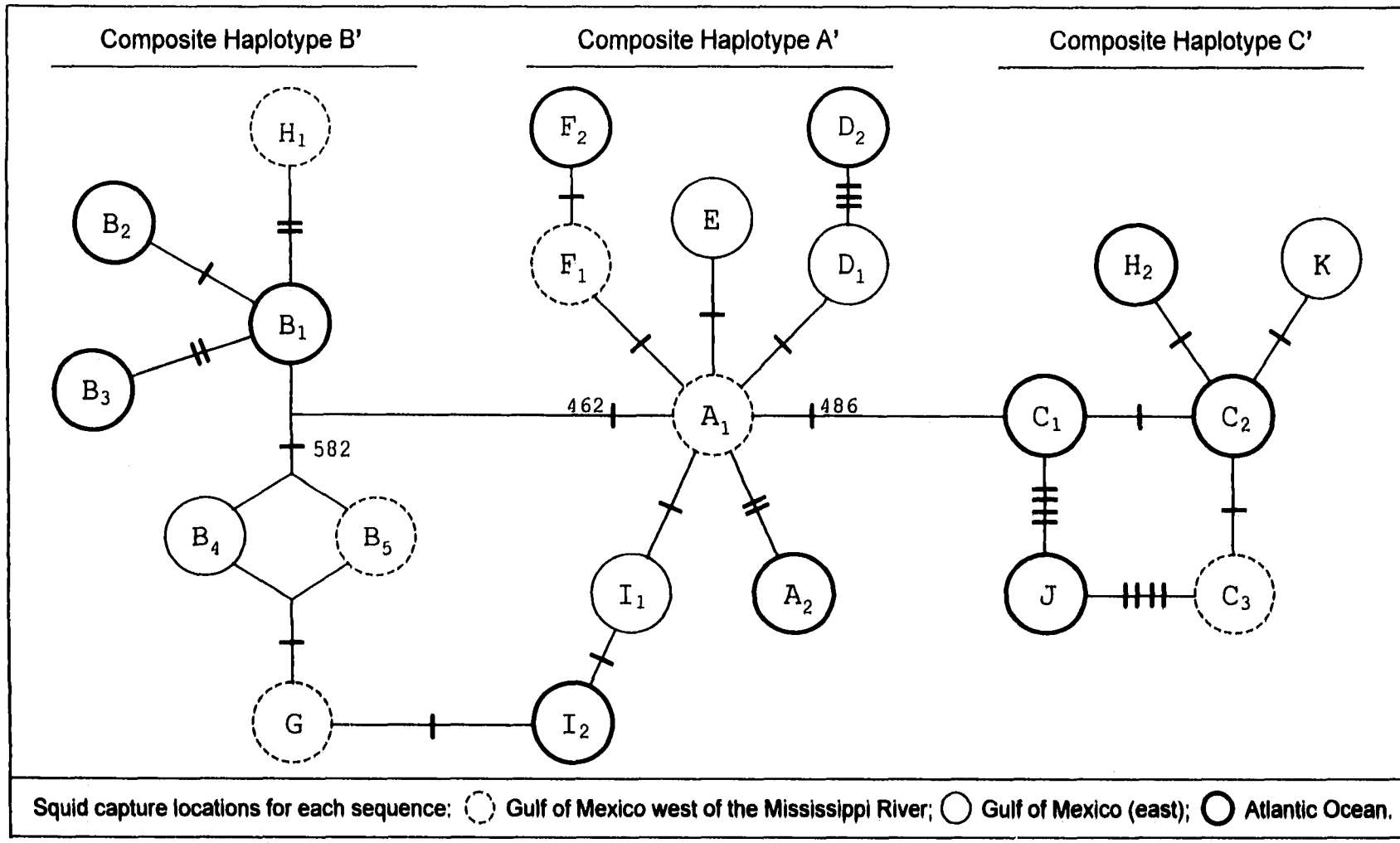

Fig. 8. *Loligo plei* (arrow squid): Minimum spanning network of CO-I nucleotide sequences for the mtDNA PCR-RFLP haplotypes (A-K, as defined in Table 1-B). Each circle represents a different DNA sequence as defined in Table 4, with nearest neighbors separated by transitions (cross-bars). Composite haplotypes A', B' and C' were used in AMOVA analyses. Variable nucleotide positions are listed for connections among the three clades. Haplotype H is composed of mtDNA lineages that have converged on the same RFLP haplotype (i.e., homoplasic lineages). Sequences B_{1-3} are the only Haplotype B specimens captured from the Atlantic Ocean.

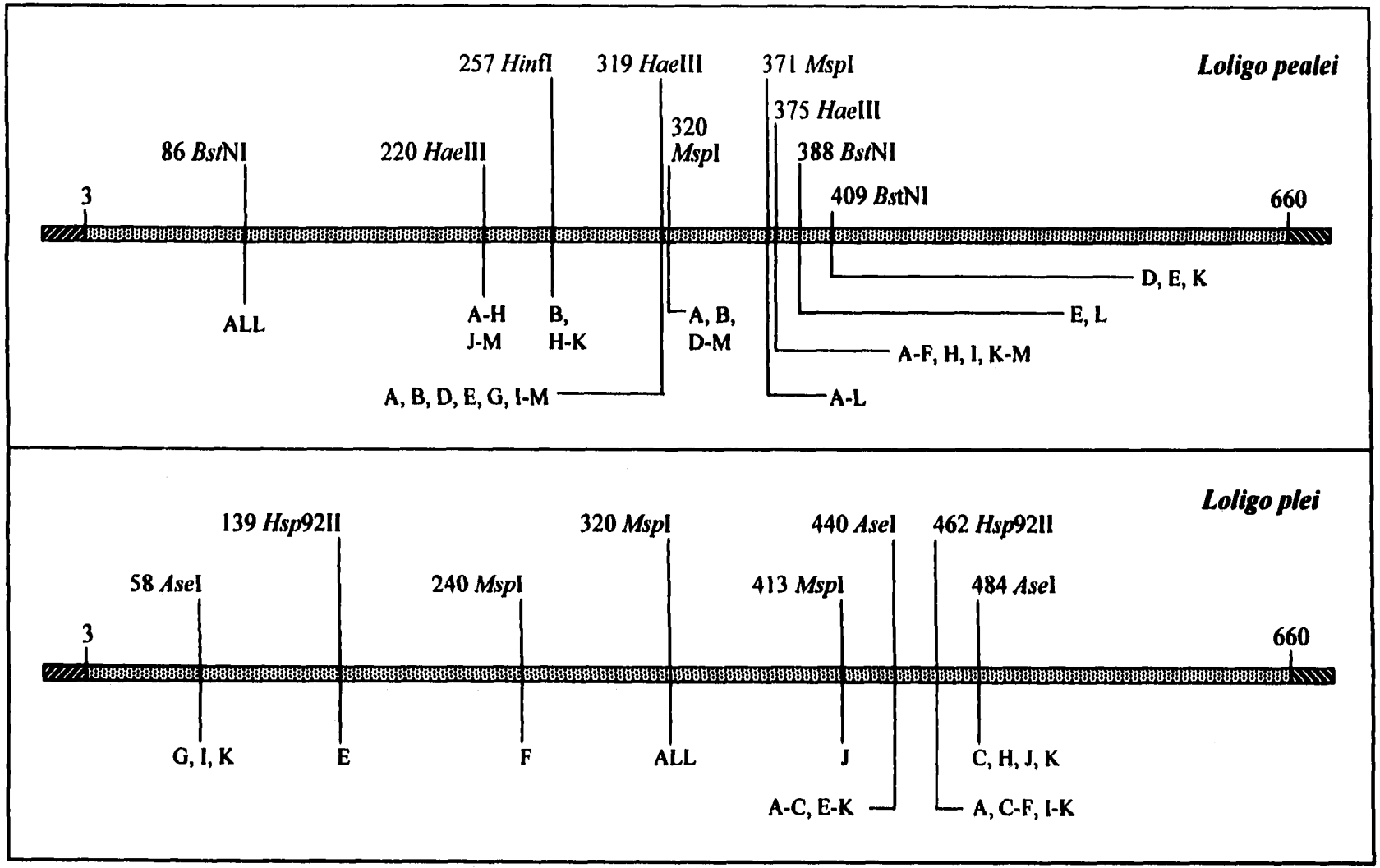

Fig. 9. Restriction sites in a 658-bp PCR product (excluding primers) of the PCR-RFLP haplotypes (mtDNA CO-I) from two species of *Loligo* squid. Restriction sites (above bar) are numbered in accordance with the complete DNA sequences shown in Figure 6; haplotypes possessing a particular restriction site are listed below the bar. L-COI primer (25 bp) = ▨; complement to H-COI primer (26 bp) = ▨.

for Haplotypes B, H-K). Restriction site absences were also homoplasic for *Hae*III (GGCC) at position 319 (GGCT for Haplotype C; AGCC for Haplotypes F and H) and at position 375 (GACC for Haplotypes G_1 and J; GTCC for Haplotype G_2). In arrow squid, absence of the *Hsp*92II (CATG) restriction site at position 462 was homoplasic for Haplotypes B, G, and H_1 (CATA) compared to Haplotype H_2 (TATG). These restriction site homoplasies might have created two instances of homoplasy in the PCR-RFLP haplotypes (i.e., convergence of DNA lineages on the same haplotype). Thus, Haplotype G in longfin squid might be polyphyletic (Fig. 7) and Haplotype H in arrow squid is clearly polyphyletic (Fig. 8).

Phylogeography: The multiple haplotypes within each species were collapsed into different composite haplotypes depending on whether the analyses were done by bar graphs or by AMOVA (Table 5). To reduce visual complexity, bar graph analyses used Haplotypes A, A*, B and B* for longfin squid and Haplotypes A, B, C, and O for arrow squid. In longfin squid, the composite haplotypes were based on the phylogenetic relationships of the less common haplotypes to the numerically-dominant Haplotypes A and B; in arrow squid, all eight rare haplotypes (12 specimens) were collapsed into the composite Haplotype O without regard for their genetic relationships. The simulation tests of AMOVA demonstrated that the apparent degree of population differentiation (even for populations that had no haplotypes in common) was reduced by the presence of either multiple haplotypes per population or low-frequency private haplotypes (i.e., haplotypes restricted to one population). Therefore, to increase the statistical power of the AMOVA analyses, longfin squid haplotypes were collapsed into Haplotypes A' and B' and arrow squid haplotypes were collapsed into Haplotypes A', B', and C' (based on the minimum-spanning networks and on Paup* analyses [not shown] for each species). Other Paup* analyses (Figs. 10 and 11) indicated that both sets of haplotypes formed essentially star-phylogenies (i.e., trees in which the relationships of the sequences cannot be fully resolved), so distance matrices were not used in AMOVA.

Table 5. Composite haplotypes of the original PCR-RFLP haplotypes (mtDNA CO-I) for two species of *Loligo*, as defined for the AMOVA analyses and the graphical analyses. Composites were based on the phylogenetic relationships seen in the minimum-spanning networks of the nucleotide sequences for representatives of each haplotype (Figures 7 and 8).

Longfin squid haplotypes			Arrow squid haplotypes		
AMOVA	Original	Graph	AMOVA	Original	Graph
A′	A	A	A′	A	A
	C	A*		D	O[a]
	D			E	
	E			F	
	F			I	
	G		B′	B	B
	L			G	O
	M			H[b]	
B′	B	B	C′	C	C
	H	B*		H	O
	I			J	
	J			K	
	K				

[a] Haplotype O is composed of 12 arrow squid with divergent haplotypes to facilitate graphing.
[b] Haplotype H (arrow squid) is composed of two individuals with homplasic DNA lineages.

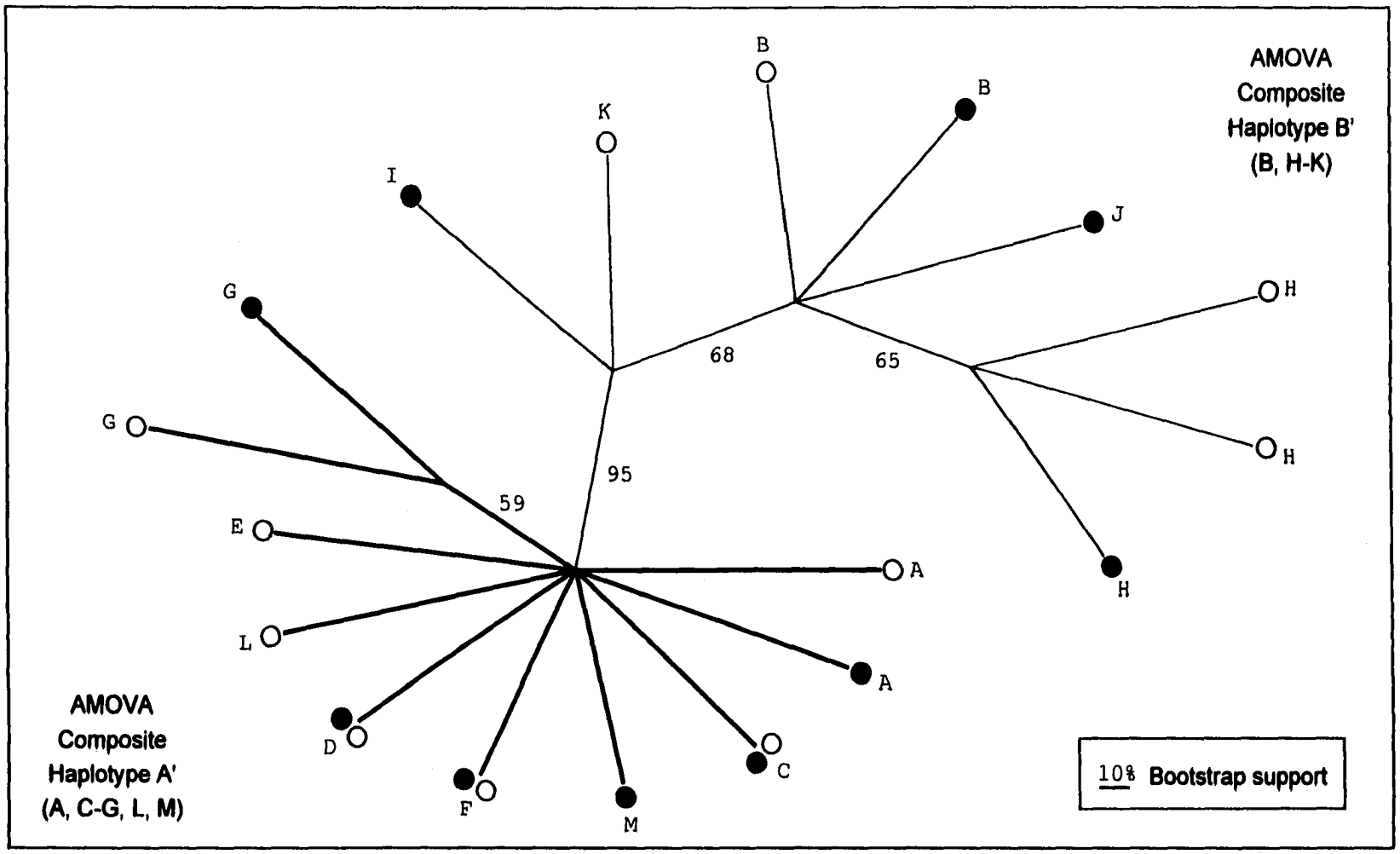

Fig. 10. *Loligo pealei* (longfin squid): Maximum-likelihood tree based on 658 bp of sequence data for the PCR-RFLP haplotypes (A-M, as defined in Table 1-A). Tree design criteria: 50% majority rule consensus tree; 100 replicates; starting trees generated by random sequence addition with 10 replicates per bootstrap replicate. LEGEND: ● for specimens captured from the Gulf of Mexico; ○ for specimens captured from the Atlantic Ocean. Line styles indicate different haplotype clades.

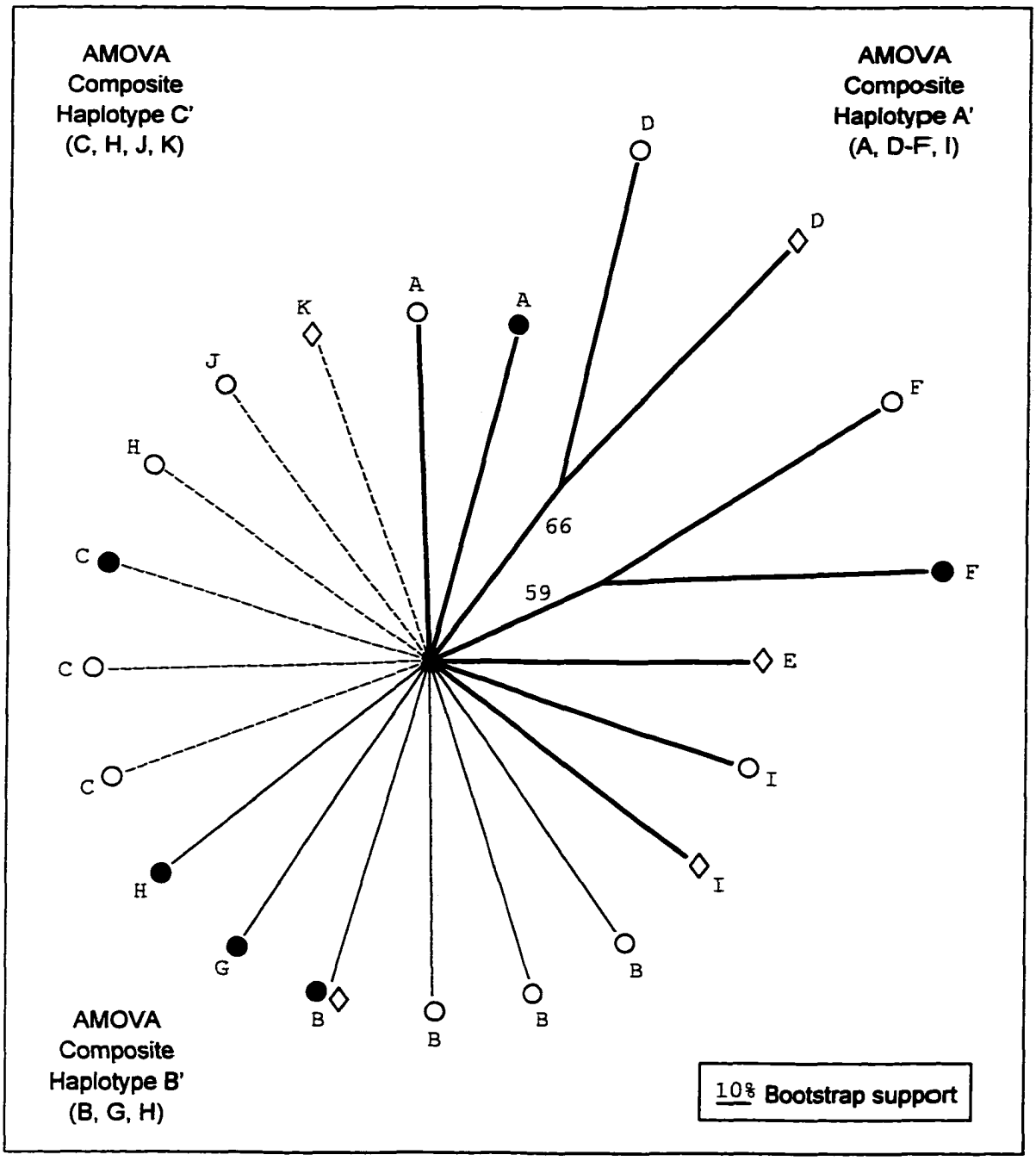

Fig. 11. *Loligo plei* (arrow squid): Maximum-likelihood tree based on 658 bp of sequence data for the PCR-RFLP haplotypes (A-K, as defined in Table 1-B). Tree design criteria: 50% majority rule consensus tree; 100 replicates; starting trees generated by random sequence addition with 10 replicates per bootstrap replicate. LEGEND: ● for specimens captured from the Gulf of Mexico west of the Mississippi River; open shapes mark specimens captured from east of the Mississippi River (◇ for Gulf of Mexico; ○ for Atlantic Ocean). Line styles indicate haplotype clades.

Bar graph analyses indicated that both species were composed of two major populations. The split for longfin squid was at the Florida peninsula, creating one population in the Atlantic Ocean and one in the Gulf of Mexico (Fig. 12). In contrast, arrow squid was split at the Mississippi River (Fig. 13), into a western population (i.e., northwestern Gulf of Mexico) and an eastern population (i.e., northeastern Gulf of Mexico and Atlantic Ocean). The relationships of the coancestry coefficients for sample units showed that gene flow within populations of longfin squid was consistent with panmixia, while gene flow within each population of arrow squid conformed to an isolation-by-distance model (Fig. 14). When the data were grouped by survey cruises (Appendix C, Table C-4), it was clear that the observed geographic patterning of haplotype frequencies was not an artifact resulting from specific surveys. There were insufficient replicate survey cruises to rigorously test for temporal changes in haplotype frequencies.

The genetic data for longfin squid were consistent with expectations for population structure in a migratory, pelagic species. Differentiation of the Atlantic samples from the Gulf of Mexico samples was due primarily to changes in the frequencies of the less common haplotypes. For instance, nearly 80% of Haplotype B occurred in the Gulf of Mexico while about 75% of the composite Haplotype A* occurred in the Atlantic Ocean. With regard to the original haplotypes, about 90% of Haplotype C and 100% of Haplotype E were found in the Atlantic Ocean (Table 3). The strength and exact placement of the phylogeographic break along the coastline of the Florida peninsula could not be determined because few longfin squid specimens were available from that area.

In contrast to longfin squid, arrow squid exhibited an abrupt phylogeographic break within the Gulf of Mexico in the vicinity of the Mississippi River. One population extended westward toward Mexico, and the other population extended eastward to Cape Hatteras in the Atlantic Ocean. West of the river, the average frequency of Haplotype A was about 30%

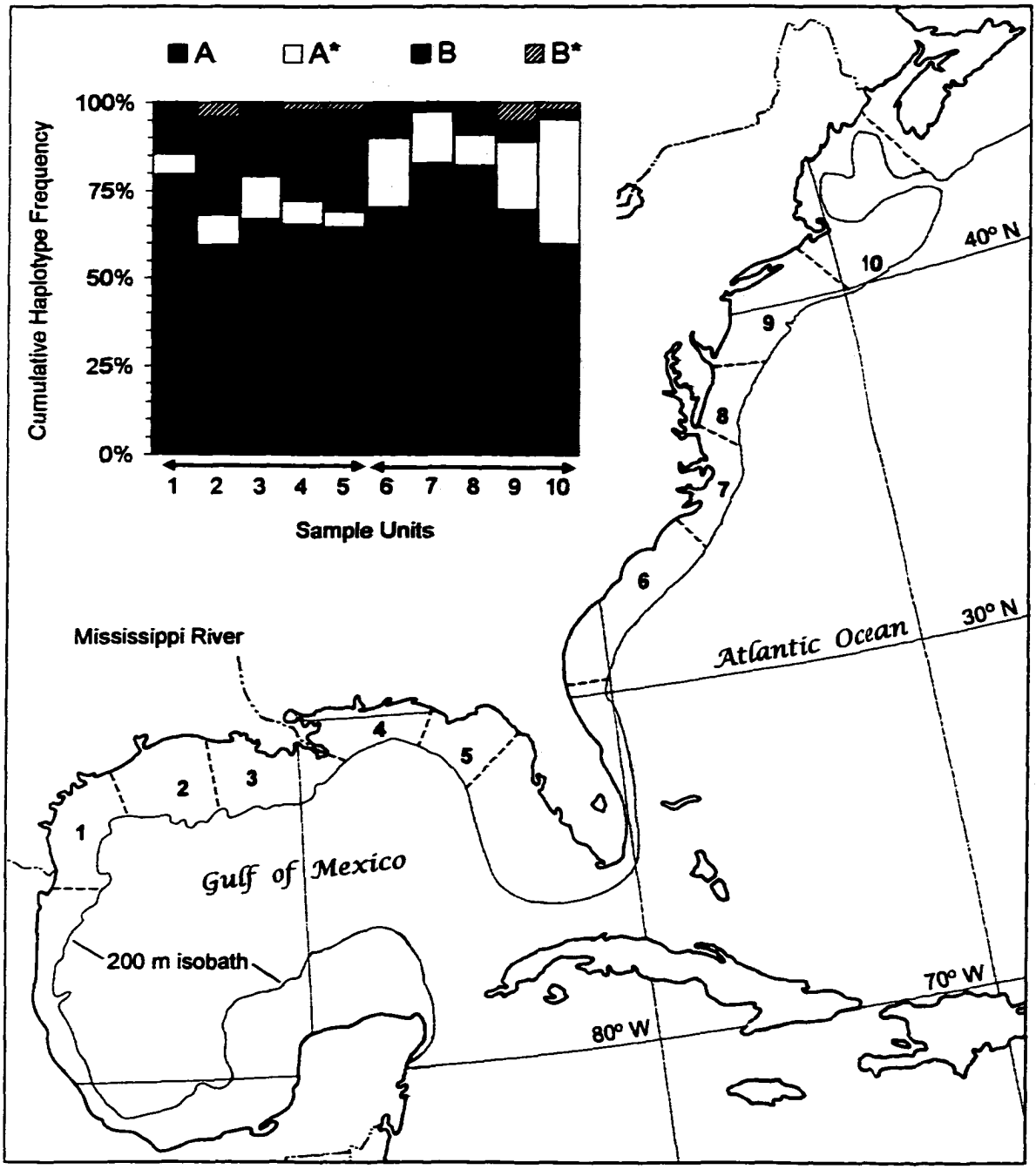

Fig 12. *Loligo pealei* (longfin squid): Overview of 10 sample units (separated by dashed lines), with their identification numbers marking the multivariate means of all trawl stations within each unit (sample size per unit: 36 squid ± 8 (SD)). Note the shift in frequencies of the composite Haplotype A* and Haplotype B between the Gulf of Mexico and the Atlantic Ocean. With regard to the statistical analyses, AMOVA Haplotype A' is composed of Haplotypes A and A*, and AMOVA Haplotype B' is composed of Haplotypes B and B* (see Table 5).

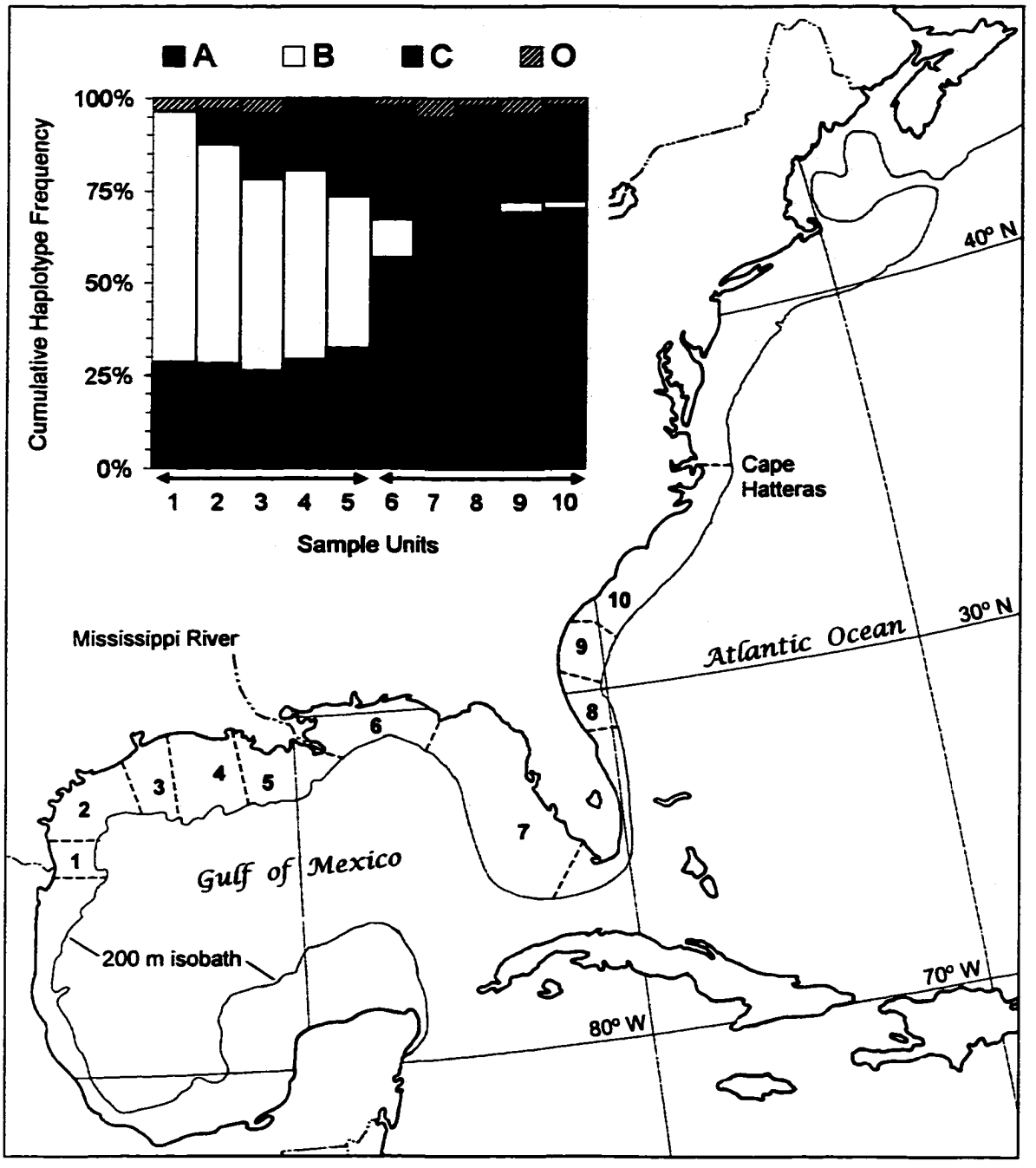

Fig 13. *Loligo plei* (arrow squid): Overview of 10 sample units (separated by dashed lines), with unit identification numbers marking the multivariate means of all trawl stations within each unit (sample size per unit: 35 squid ± 8 (SD), excluding unit 6 [$n = 86$] and unit 9 [$n = 68$]). Note the shifts in haplotype frequencies west versus east of the Mississippi River. Haplotype O is a composite of 12 individuals (eight rare haplotypes; see Table 5).

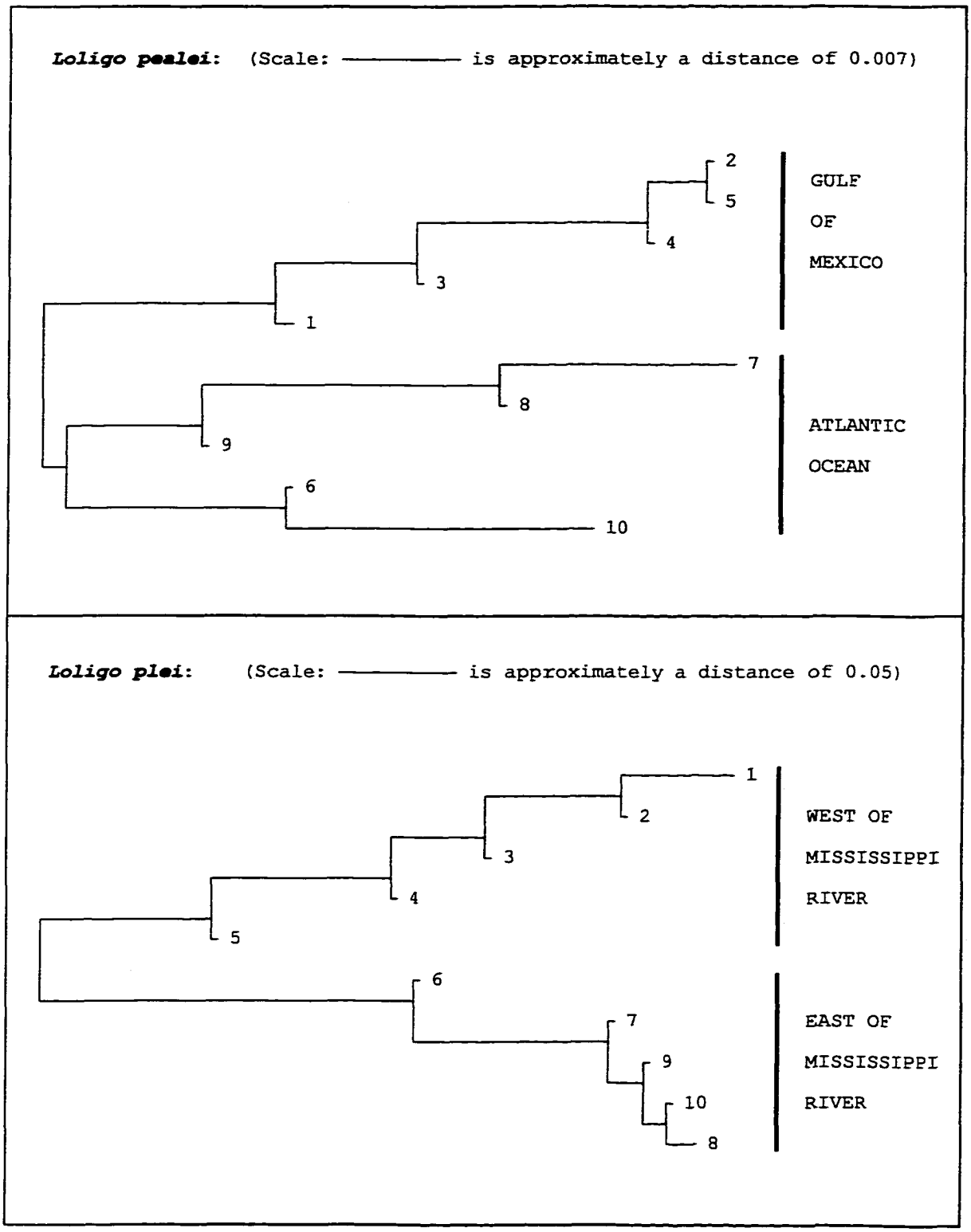

Fig. 14. **Neighbor-joining relationships of sample units based on their coancestry coefficients (from AMOVA, using all haplotypes). For each species, sample unit 1 is the most western location in the Gulf of Mexico and sample unit 10 is the most northern location in the Atlantic Ocean.**

across all sample units, whereas Haplotype C gradually decreased from 25% to 0% and Haplotype B slowly increased from 40% at the river to 70% near Mexico. All samples east of the river were dominated by Haplotypes A and C; average frequencies were approximately 70% A, 25% C, and 5% rare types, with the exception of sample unit A-6 which had about 10% Haplotype B (nine specimens). Eight of these Haplotype B specimens were captured south of Mobile Bay, Alabama (30°N and 88°W), and the representative DNA sequence was identical to that of a Haplotype B specimen captured off central Texas (sample unit A-3); the three Atlantic Ocean specimens with this haplotype all had unique DNA sequences.

The AMOVA analyses supported the population subdivisions noted above for both species (Tables 6 and 7). Within their putative populations, there was no discernible genetic structure for either longfin squid ($P > 0.52$) or for arrow squid ($P > 0.29$). In contrast, the "Among Groups" percentage of haplotype variance (σ_{AG}) was significant ($P < 0.008$; AMOVA permutation value) for both species: $\sigma_{AG} = 11.2\%$ for longfin squid; and, $\sigma_{AG} = 29.5\%$ for arrow squid. The σ_{AG} significance values are conservative because lower probabilities are impossible given that there are only 126 unique ways to combine 10 sample units into two groups ($1/126 = 0.008$); further, the σ_{AG} remained significant for both species ($P < 0.02$) even after being adjusted for multiple tests (i.e., three hierarchal levels within each species).

In addition, strong population subdivision was indicated by significant F_{st} values ($F_{st} = 0.112$ for longfin squid; and, $F_{st} = 0.295$ for arrow squid). F_{it} values were also significant for both species. Given that F_{it} is approximately equal to $F_{is} + F_{st}$, either F_{is} or F_{st} would be significant in these data sets no matter what geographic partitions were chosen for the AMOVA analyses. Thus, the F_{st} values are not significant simply because of having tested the geographic partitions with the greatest differences in haplotype frequencies.

For arrow squid, the initial haplotype frequencies of sample unit A-6 suggested that a phylogeographic break existed at longitude 88°W (between Mobile Bay and Pensacola Bay), so

Table 6. *Loligo pealei* (longfin squid): Results of the AMOVA (Analysis of Molecular Variance – Arlequin version 1.1), based on frequencies of PCR-RFLP Haplotypes A' and B' (mtDNA CO-I). Groups are populations in the Gulf of Mexico and the Atlantic Ocean.

Source of variation	d.f.	Sum of squares	Variance (σ) component	σ (%)	σ (*P*)	Fixation indices
Among Groups	1	2.91	0.0158	11.2	**	F_{st} = 0.112
Among Units within Groups	8	0.90	-0.0004	-0.3	n.s.	F_{is} = -0.003
Within Units	346	43.39	0.1254	89.1		F_{it} = 0.110
Total	355	47.19	0.1408	100.0		

** $P < 0.02$ as corrected by a sequential Bonferroni procedure to account for multiple tests; original significance values were based on the probability of finding by chance a more extreme variance component through permutations.

Table 7. *Loligo plei* (arrow squid): Results of the AMOVA (Analysis of Molecular Variance – Arlequin version 1.1), based on the frequencies of PCR-RFLP Haplotypes A', B', and C' (mtDNA CO-I). Groups are populations East and West of the Mississippi River.

Source of variation	d.f.	Sum of squares	Variance (σ) components	σ (%)	σ (*P*)	Fixation indices
Among Groups	1	20.66	0.1046	29.5	**	F_{st} = 0.295
Among Units within Groups	8	2.61	0.0018	0.5	n.s.	F_{is} = 0.007
Within Units	421	104.59	0.2484	70.0		F_{it} = 0.300
Total	430	127.86	0.3548	100.0		

** $P < 0.02$ as corrected by a sequential Bonferroni procedure to account for multiple tests; original significance values were based on the probability of finding by chance a more extreme variance component through permutations.

more samples were analyzed from those areas (Table 8). Final haplotype frequencies of the Mobile Bay and Pensacola Bay subunits were significantly different by the log likelihood ratio, $G_2 = 21.3$ ($P < 0.001$). Yet, the frequencies were also significantly different between Mobile Bay and the western sample unit A-5 ($G_2 = 10.8$, $P < 0.01$) as well as between Pensacola Bay and the eastern sample unit A-7 ($G_1 = 4.6$, $0.025 < P < 0.05$).

Neither different sampling times nor an unusual trawl catch explained the aberrant distribution of haplotypes in this area. First, all but one of the 86 squid were captured between 6 and 10 November 1995. Second, squid were drawn equally from five trawl stations south of Mobile Bay and from four trawl stations south of Pensacola Bay; further, the haplotype frequencies at each station were consistent with the other trawl stations in the same area. Upon merging the two sample areas to create sample unit A-6, the haplotype frequencies were not significantly different from those of the eastern sample unit A-7 ($G_2 = 3.1$, $P > 0.05$); in contrast, the frequencies of sample unit A-6 were significantly different from those of the western sample unit A-5 ($G_2 = 14.2$, $P < 0.001$). The Bonferroni corrections for multiple tests ($P < 0.05$) did not eliminate any of the significant differences above, except for the comparison of the Pensacola Bay subunit with sample unit A-7 (which was reduced to nearly significant).

Table 8. Arrow squid (*L. plei*) haplotype frequencies for testing a potential phylogeographic break between Mobile Bay and Pensacola Bay in the northern Gulf of Mexico.

Sample units	*n*	Haplotype Frequency (i.e., as a % of *n*)		
		A	B	C
Mobile Bay subunit	40	70[a]	20	10
Pensacola Bay subunit	46	48	2[b]	50[c]
A-5 (western population)	34	32	42	26
A-6 (eastern population)	86	58	10	32
A-7 (eastern population)	37	72	0[d]	28

[a] Value includes one specimen of Haplotype I.
[b] Grouped with Haplotype A in comparison of Pensacola Bay with sample unit A-7.
[c] Value includes one specimen of Haplotype K.
[d] One hypothetical specimen was used for comparison of sample units A-6 and A-7 (G-test cannot be done if one area lacks a haplotype, because the "Ln 0" is undefined).

DISCUSSION

For both longfin squid and arrow squid, the broadest conclusion of this study is that the data reject the null hypothesis of genetic homogeneity (i.e., F_{st} was not zero) within North American waters. However, while both species were composed of two populations, their phylogeographic patterns were not similar. Longfin squid displayed the classic pattern of Gulf and Atlantic populations (reviewed in Avise, 1996); on the other hand, arrow squid populations were separated at the Mississippi River such that the eastern population encompassed nearly half of the Gulf of Mexico as well as the Atlantic. Further, patterns of gene flow were dissimilar between the species. Within populations of longfin squid, gene flow was consistent with panmixia. For arrow squid, moving both east and west from the Mississippi River, gene flow was consistent with an isolation-by-distance model (i.e., adjacent sample units were more closely related to each other than to more distant sample units).

Phylogeographic Patterns: Concerns have been raised by Grant et al. (1998) regarding the use of RFLP analysis versus direct sequence analysis for examining phylogeography. For instance, different biogeographic connections were postulated for five regional populations of the sardine (*Sardinops*), depending on whether the genetic data came from an RFLP analysis of the mtDNA control region and flanking regions (2 kb) or a sequence analysis of only the control region (500 bp). The conflict was ascribed to the inability of an RFLP analysis to distinguish nucleotide transitions from transversions as well as to detect homoplasy within restriction sites. The objections regarding homoplasy are not pertinent to this study. When two DNA sequences are compared, the evolutionary independence of nucleotides at the same position is unambiguous only when the nucleotides are different. Therefore, DNA sequencing will detect homoplasy in RFLP analyses only in those instances when the absence (not presence) of a restriction site created homoplasic RFLP haplotypes. Within each species of *Loligo*, homoplasic restriction site absences were evident from the CO-I sequences of each

RFLP haplotype. Some homoplasies occurred through multiple-substitutions at the same nucleotide position, while others were generated by base changes at different positions within the same restriction site. However, at the level of the RFLP haplotype, the only adverse effect was to create a potentially polyphyletic Haplotype G in longfin squid (Fig. 7) and a clearly polyphyletic Haplotype H in arrow squid (Fig. 8) – a total of four individuals. Based on the representative sequences of all haplotypes, it is unlikely that more DNA sequencing would have detected additional RFLP homoplasies (for the enzymes used) in either species. Further, simply sequencing CO-I for every individual would not clarify the phylogeography of these squid. In the maximum-likelihood trees generated by Paup* (Figs. 10 and 11), the sequences did not cluster geographically and there was little bootstrap support for any of the branches (except for between Haplotypes A' and B' in longfin squid). For CO-I sequence data on all specimens to be useful, it would have to be filtered for specific nucleotide positions that were geographically-informative; the filtering process can be done automatically through an RFLP analysis.

None of the intraspecific nucleotide substitutions produced any inferred amino acid replacements within either species. Despite the large interspecific DNA sequence divergence, arrow squid were distinguished from longfin squid by only two predicted amino acid replacements. Such low protein-level divergence (0.9%) is in keeping with the strong evolutionary constraints on the function of the CO-I protein (Simon et al., 1994). Similarly, Carlini and Graves (1999) found a 0.98% mean amino acid sequence divergence in CO-I for Oegopsida – the sister suborder to Myopsida (which includes *Loligo*).

The nucleotide divergence rate of CO-I has been estimated at 1.4% per million years in a marine crustacean (*Alpheus* spp.); the estimate was based on 15 pairs of sister-species which were separated by the emergence of the Isthmus of Panama about three million years ago (Knowlton & Weigt, 1998). Assuming the CO-I divergence rate is similar for cephalopods,

longfin squid haplotypes would coalesce to Haplotypes A and B within about 100,000 years and to a common ancestral haplotype within 700,000 years. All of the common arrow squid haplotypes would coalesce within about 100,000 years. These time frames predate the last glacial period in North America (falling within the Pleistocene epoch), while the estimates for interspecific nucleotide divergence suggest the two species originated approximately 10 million years ago during the Miocene epoch (Briggs, 1995). Further, the common haplotypes of longfin squid appear to be older than those of arrow squid by an order of magnitude, but this might be a sampling artifact related to the study area having included the primary range of the temperate longfin squid as compared to the peripheral northern range of the tropical arrow squid. In any event, all intraspecific haplotypes are of recent origin in comparison to the species lineages. Such shallow population histories embedded in deep evolutionary lineages are common for marine taxa (Billington & Hebert, 1991; Grant et al., 1998; Graves, 1998). Explanations for this phenomenon range from demographic events (e.g., bottlenecks, regional extinctions, and secondary contact) to stochastic loss of female mtDNA lineages (accelerated by fluctuations in abundance and variance in reproductive success).

Causes of Phylogeographic Differences: The different phylogeographic patterns observed in these two cephalopods are likely due to a combination of their respective ranges as well as differences in their salinity and temperature tolerances. Measuring along the continental shelf, the complete range of longfin squid covers about 11,000 km of coastline (5,000 km in North American waters). Migrations by this species appear restricted to following the continental shelf because specimens are rarely found even at oceanic islands that are located close to the continents (Cohen, 1976). Hence, the only possible migration route between the Gulf and Atlantic populations of longfin squid is around the southern tip of Florida. In contrast, the range of arrow squid extends across 19,000 km of the continental shelf (3,500 km in North American waters), and this species also occurs throughout the Caribbean

(Moynihan & Rodaniche, 1982). Thus, genetic connections for arrow squid might be complex. For instance, Cuba and the Yucatan Peninsula might provide a link between the eastern population of arrow squid and the southwestern Gulf of Mexico. Further, the eastern population might be connected genetically to South American arrow squid by the Grand Bahama Bank and the Antilles (Voss & Voss, 1960), a link of approximately 2,500 km.

Salinity and temperature tolerances probably play roles in the bathymetric distributions of these two species. Where their ranges overlap (i.e., from 35°N to 10°N), longfin squid dominates the outer continental shelf (between the 40 m and 200 m isobaths) while arrow squid dominates the midshelf (between the 20 m and 40 m isobaths); however, it is not unusual to capture both species at the same site (Cohen, 1976; Hixon et al., 1980; H. Perry, Gulf Coast Marine Research Laboratory, Ocean Springs, MS, personal communication). Longfin squid are rarely found in salinities of less than 33‰ while arrow squid will venture into salinities as low as 30‰ (Hixon et al., 1980). As seen in the data compilations of Voss and Brakoniecki (1985), surveys usually find few specimens of either species at the discharge point of major rivers (e.g., the Mississippi River), although laboratory studies show that both species can survive for several days at salinities lower than 30‰ (e.g., Hanlon et al., 1983). With respect to temperature, the longfin squid is found in waters that are 9°C to about 22°C; in contrast, the tropical arrow squid is found over a temperature range of about 12°C to 30°C.

The influence of temperature on these species is apparent in its effects on their annual migration patterns (Arocha & Urosa, 1991; Costa & Fernandez, 1993; Summers 1983; Whitaker, 1978). North of Cape Hatteras where surface waters fall below 8°C during winter, longfin squid overwinter in canyons on the margins of the continental slope and then migrate inshore and northward during early summer when the surface waters warm sufficiently. South of Cape Hatteras (Atlantic Ocean) and off Venezuela (Caribbean Sea) where temperatures exceed 22°C during the summer, longfin squid reverse the migration pattern and move closer to

shore when surface waters cool during winter. In the Atlantic, arrow squid are present south of Cape Hatteras throughout the year, although only small squid remain during the winter (Whitaker, 1978). In the northern Gulf of Mexico, arrow squid are restricted to south Florida during the winter according to Voss and Brakoniecki (1985); however, it is likely that most of the surveys upon which their assessment was based were unable to distinguish small arrow squid from longfin squid.

Curiously, in the northwestern Gulf of Mexico, Hixon (1980) found that longfin squid moved inshore in spring and offshore in autumn. In contrast, numerous longfin squid were found in the nearshore waters of Texas by an October-November 1995 survey of the northern Gulf of Mexico (some specimens used in the present study came from that survey). However, it is unclear which species of *Loligo* was captured because the average mantle length was only 5 cm (from all subsamples, a total of 1,174 squid were measured). Such tiny squid are difficult to identify; further, many were reclassified as arrow squid based on the mtDNA results of the present study. What is clear from the survey data is that nearly 90% of the squid taken in that autumn cruise were small ($\bar{x} = 4$ cm $\pm$ 2 SD), and over 70% of the larger squid ($\bar{x} = 16$ cm $\pm$ 6 SD) were taken east of the Mississippi River. It is unknown whether most of the larger squid had died or migrated south to warmer waters. For the eastern arrow squid, suitable overwintering areas might include either southern Florida (Voss & Brakoniecki, 1985) or the Bahamian Banks and the Antilles of the Caribbean Sea; for the western population, arrow squid might overwinter in the Bay of Campeche off southern Mexico.

The above factors in combination with the oceanographic features of the region appear sufficient to explain the different phylogeographic patterns of these two cephalopods. With respect to longfin squid, samples from south Florida are required to determine whether the phylogeographic break in this species is strictly Gulf versus Atlantic or whether it falls at an intermediate location (e.g., at Cape Canaveral which is a point of biogeographic divide for

tropical and temperate fauna in the Atlantic). Nevertheless, a case can be made for the strict separation scenario. First of all, the Straits of Florida lie between the Gulf of Mexico and the Atlantic Ocean, with the flow of water being primarily eastward. Further, the continental shelf south of Florida is narrow and surface water temperatures typically exceed 24°C, being derived from the South Atlantic. Although suitable temperatures (< 22°C) for longfin squid exist along the narrow band of continental shelf between 100 m and 400 m (the maximum depth recorded for this species), currents in the Straits of Florida range from 20 to 50 cm/s, sometimes even exceeding 100 cm/s (Lee et al. 1994). Such currents would prevent passive distribution of paralarvae from Atlantic longfin squid into the Gulf of Mexico. Even adult squid would likely have difficulty making the passage into the Gulf. Indeed, the present data suggest that gene flow between the Gulf and Atlantic populations is primarily eastward through the Straits. For instance, Haplotypes C and E were essentially restricted to the Atlantic and yet Haplotype B, while more common in the Gulf, occurred at appreciable frequency throughout the Atlantic. Given the migratory abilities of longfin squid, this pattern of gene flow is more consistent with a phylogeographic break at south Florida than at Cape Canaveral.

With respect to the classic Atlantic-Gulf split seen for longfin squid and for many other marine taxa (Avise, 1992, 1996), arrow squid showed no evidence of a phylogeographic break. Instead, a break was evident at the Mississippi River in the center of the northern Gulf of Mexico. The Mississippi River drains about two-fifths of the U.S. and discharges freshwater over the continental shelf; therefore, the relative proximity of the two species to the shore might explain their different responses to this putative barrier. In the northern Gulf of Mexico, the continental shelf is very wide (except for immediately at the current discharge location for the river); as a result, longfin squid are found farther offshore than are arrow squid. Given that longfin squid inhabit cooler, high salinity, and relatively deep waters in the Gulf, that species can probably swim around the freshwater discharge of the Mississippi River.

However, the arrow squid inhabits shallower, warmer waters and avoids low salinity waters, so the Mississippi River discharge might present a substantial barrier to longshore movement by this species. In contrast, the Florida peninsula is unlikely to present a barrier to arrow squid. First of all, the Loop Current (part of the Gulf Stream) should bring squid paralarvae from the eastern Gulf into the Atlantic. Second, arrow squid are more tolerant of high temperatures than are longfin squid; thus, they can avoid the strong, eastward currents of the Florida Straits by swimming westward through the saline waters of the Florida Keys. Finally, the Atlantic arrow squid are at the most northern range of the species and might simply be an annual extension of the population from the eastern Gulf of Mexico or the Caribbean Islands.

The aberrant samples of arrow squid from south of Mobile Bay and Pensacola Bay could represent local endemism, perhaps maintained by factors related to the area being bounded on the west by the freshwater discharge of the Mississippi River and on the east by the extension of the Loop Current. This suggestion of endemism is supported by the observation of a high frequency of endemism for freshwater fishes and mollusc species in the Florida panhandle region (references in Katoh & Foltz, 1994). However, it seems improbable that a wide-spread, highly mobile marine organism such as the arrow squid would have such locally endemic populations. Instead, it seems likely that the Mobile-Pensacola area represents a temporary mixing zone between the western and eastern populations. In support of this hypothesis, Haplotype B occurred only 12 times in the eastern population (i.e., three times in the Atlantic Ocean and nine times in the Gulf of Mexico); yet eight of the eastern Gulf squid were taken from due south of Mobile Bay, Alabama, immediately to the east of the Mississippi River. Further, of the five sequenced representatives of Haplotype B, the two squid from sample units A-3 and A-6 had identical nucleotide sequences, while the three specimens in the Atlantic all had unique sequences. More sequencing is needed to document the close relationship of all Gulf specimens possessing Haplotype B, but it appears that the specimens

found east of the Mississippi River (in the Gulf) belong to the western population. In contrast, the rare squid in the Atlantic with Haplotype B probably derive from an ancestor to the western Haplotype B.

Alternate Hypotheses: In the context of evolutionary time, the freshwater discharge of the Mississippi River is likely to represent an intermittent barrier to arrow squid. As such, it seems improbable that the western and eastern populations have been maintained *in situ* for thousands of years. Several other scenarios would be consistent with the current data.

- H_A-1: Secondary contact, following population expansion, has occurred after a previously homogeneous population of arrow squid collapsed to form small, isolated populations in the western and the eastern Gulf of Mexico.

It has been argued that the biological instabilities of the squid life cycle inevitably lead to cyclic population collapses (O'Dor & Coelho, 1993). This scenario suggests that squid exhibit metapopulation dynamics (episodic extinctions and recolonizations), similar to the situation proposed by Bowen and Grant (1997) to explain the world-wide biogeographic patterns of the sardine, *Sardinops*. If a major collapse in the recent past left isolated populations of arrow squid on either side of the Mississippi River, then stochastic loss of haplotypes could have generated the current haplotype frequencies in each of the new populations. As the two populations recolonized the northern Gulf of Mexico, secondary contact would eventually occur at the Mississippi River.

- H_A-2: Annual recolonization of the northern Gulf of Mexico and the northwestern Atlantic Ocean occurs through immigration from more southern arrow squid populations.

Gene flow in arrow squid was consistent with an isolation-by-distance model (Fig. 14), with the Mississippi River being the focal point for both populations. This suggests that the northern Gulf of Mexico and the northwestern Atlantic might be recolonized annually by arrow squid from more southern populations. Eastward migration from the southwestern Gulf and

westward migration from south Florida or the Caribbean Islands would be slowed, but not necessarily stopped, when the Mississippi River was reached. Consistent with this hypothesis was that 75% of the Haplotype B specimens found in the eastern population were located south of Mobile Bay. Although the Florida Straits lie between the islands and Florida, the strong eastward currents are not necessarily a major barrier to squid attempting to migrate north. For instance, a gyre 200 km in diameter forms several times a year off the Dry Tortugas (north of the western end of Cuba), with each episode lasting up to three months. This Tortugas Gyre has been implicated as a factor in retaining fish larvae on the southwest continental shelf of Florida (Lee et al., 1994). Presumably, the gyre would also help arrow squid cross the Florida Straits. Further, even arrow squid swept around eastern coast of Florida could subsequently migrate back into the northeastern Gulf through the Florida Keys.

- H_A-3: The distribution of the carbonate sediments of the Florida escarpment is an ancillary factor for the phylogeographic pattern of arrow squid, while the freshwater discharge of the Mississippi River is the primary factor.

Under either the secondary contact hypothesis or the annual recolonization hypothesis, the Mississippi River appears to be the primary factor that separates the western and eastern populations of arrow squid. However, an ancillary factor could be that carbonate sediments comprise the Florida escarpment and the western edge of the escarpment lies in the Pensacola Bay region (29°N, 88°W). To the west lie organic-laden muds, which owe their origins at least in part to deposition by the Mississippi River (Wilhelm & Ewing, 1972). The boundary between these two types of sediment also marks the boundary between the arrow squid samples from the Mobile Bay and Pensacola Bay subunits (in sample unit A-6). The significant difference in haplotype frequencies between the two subunits could be due to natural selection operating differently on the western and eastern populations. For instance, to hide from predators, squid will take on the appearance of the substrates; not only do squid match the

substrate colors, but they also change their textural appearance to blend with the substrate (Hanlon & Messenger, 1996). Perhaps the populations of arrow squid are better adapted to blending with the substrates found in their region, leading to higher survival rates for members of each population in areas with the proper substrates. While it is unlikely that the mtDNA haplotypes would be directly selected, the observed differences could result from lineage-sorting of the mtDNA haplotypes (Hare, 1998). Alternatively, arrow squid might simply key into the type of substrate to which they are accustomed, such that they generally avoid crossing the boundary between the sand-mud habitats.

- H_A-4: The distribution of the carbonate sediments of the Florida escarpment as compared to the organic-laden muds of the western Gulf of Mexico is solely responsible for the phylogeographic pattern of arrow squid.

Under this hypothesis, it is purely coincidental that the mouth of the Mississippi River lies in the vicinity of the phylogeographic break observed for arrow squid. Instead, benthic-related factors are the primary cause of the break. For arrow squid, this hypothesis is consistent with the strong partitioning of the haplotype frequencies seen between the Mobile Bay and Pensacola Bay subunits. However, it is at odds with the substantial increase in the frequency of Haplotype B immediately to the west of the river. Further, longfin squid showed no phylogeographic response to the change in sediment, although the influence of the Florida escarpment on that species might be reduced given that longfin squid are typically found in deeper waters located farther offshore than is true for arrow squid.

The present data cannot conclusively distinguish among these four hypotheses. However, additional arrow squid samples from the Bahamas, the Antilles, and the southwestern Gulf of Mexico would demonstrate whether the annual recolonization hypothesis is more plausible than the secondary contact hypothesis. Further, all of these hypotheses can be evaluated by determining the population genetic structure of the region's third common

loliginid, the brief squid (*Lolliguncula brevis*). The brief squid inhabits brackish (17‰) to saline (34‰) environments from 39°N (Delaware Bay) to 23°S (Sao Paulo, Brazil). Further, it is restricted to inshore waters and shallow bays (< 20 m deep). Because the brief squid tolerates low salinities, the Mississippi River should not present a significant barrier to gene flow in this species. Hence, concordance of phylogeography within the northern Gulf of Mexico for the arrow squid and the brief squid would support the sand-mud sediment hypothesis. In contrast, the lack of a strong break for the brief squid east of the Mississippi River would be consistent with the hypothesis that the discharge from the river generates a low salinity barrier for arrow squid. In addition, the more inshore brief squid ranges much farther north than arrow squid, so a study of gene flow within brief squid populations could clarify likelihood of two-way movement by arrow squid around the Florida peninsula.

Fisheries Management: Squid are a major component of the marine ecosystem, providing a link between smaller prey items and larger fishes, marine mammals, and birds (Carvalho & Loney, 1989). With the decline of many finfish fisheries, expanding squid populations have been targeted by commercial fishermen in recent years (Pierce & Guerra, 1994). For instance, members of the family Loliginidae (Cephalopoda: Suborder Myopsida) constitute several major fisheries around the world, accounting for about 9% of the world cephalopod catch (Roper et al., 1984).

Overall, the typical life history characteristics of cephalopods makes them prone to explosive population expansions and contractions (O'Dor & Coelho, 1993). Directed fisheries could exacerbate population crashes. Also, for such annual semelparous species, strong selection pressure resulting from long-term, intensive exploitation might reduce average body size at maturity, thereby diminishing the reproductive potential of the species. This could lead to reduced genetic variation and compromise resilience to environmental fluctuations (Brodziak & Macy, 1996; Murphy et al., 1994). As such, it is important to characterize the

population structures of longfin squid and arrow squid throughout their ranges prior to further significant exploitation. Otherwise, inappropriate management decisions are inevitable and genetically-distinct populations of these species are likely to be imperiled.

Prior to the 1960's, squid fisheries along the Atlantic coast of North America were small, domestic operations (Brodziak, 1998). Since then, longfin squid has supported a major fishery in the northeastern U.S., with annual landings averaging 17,300 metric tons during 1963 – 1992 (Brodziak & Macy, 1996); however, there are no U.S. fisheries that target either *Loligo* or *Lolliguncula* farther south. Nevertheless, all three species occur as bycatch in other fisheries (such as for shrimp), and are sometimes processed for sale as bait or for local consumption (Hixon et al., 1980; Horst, 1986; Roper et al., 1984; G. Adkins, Louisiana Department of Wildlife and Fisheries, personal communication). In southern U.S. waters, the typical maximum size (mantle length) for both *Loligo* species is about 15 – 25 cm, while *Lolliguncula* has a maximum size of about 8 – 10 cm (Hixon, 1980; LaRoe, 1967). Prior surveys of squid biomass in the southeastern U.S. and in the northern Gulf of Mexico suggested that squid populations were too low to support major fisheries (Hixon et al., 1980; Voss et al., 1973; Whitaker, 1980). In contrast, based on compilations of data records from government surveys and commercial fisheries, Voss and Brakoniecki (1985) suggested that these regions could support commercial squid fisheries. Further, even if the biomass of squid is low in southern U.S. waters, recent changes in commercial fishing might create interest in these squid populations as a means to supplement incomes.

For instance, many of the groundfish populations in the Northeast have collapsed and are unlikely to recover for many years (Canfield, 1997; Fraser, 1999). The Northeast U.S. squid fishery recently switched to a limited-entry permit system because the fishery was already fully exploited and it was likely to face increased pressure from fishermen who formerly targeted groundfish (Brodziak, 1998). Lacking other alternatives, some of those

fishermen might move south to tap unused and currently unmanaged squid populations. Also, another unemployed group of fishermen was created recently in Florida, Louisiana, and Texas when traditional gill-net fisheries were largely banned within state waters. Finally, commercial fishermen claim that requirements for turtle and finfish exclusion devices have made trawling for shrimp (*Penaeus* spp.) less profitable in U.S. waters (Trunk, 1998). During slack shrimping times, these fishermen could target squid instead.

As these and other fisheries continue to decline and as new regulations and legislation shrink existing fisheries, species which previously were economically unfavorable might become targets of opportunity from many sources. Assuming that squid biomass is low south of Cape Hatteras, local consumption might be required to make fishing for squid profitable. Nevertheless, imported squid can already be found in southern U.S. supermarkets and demand for locally captured squid could be increased by aggressive marketing (Hixon et al., 1980).

Consequently, this research has implications for management of loliginid fisheries within the northwestern Atlantic Ocean and the northern Gulf of Mexico. For instance, out of 180 squid taken from north of latitude 34°N, only five (< 3%) were arrow squid, and all five squid were taken at 36°N; these results are consistent with those of Cohen (1976). Thus, it can be assumed that nearly all *Loligo* north of Cape Hatteras are longfin squid, but to the south, managers will have to deal with the presence of both species. Additional research is needed to determine if directed fisheries can target the two species separately given that they are sometimes found within the same bathymetric depths. The situation will be further complicated by the similar physical appearance of juvenile *Loligo*, although only minimal training is required for distinguishing larger specimens (> 10 cm mantle length) of longfin squid from arrow squid on the basis of external morphology.

There is clearly evidence for at least two stocks within both longfin squid and arrow squid. However, as noted by Pawson and Jennings (1996), genetic stock analyses are valuable

when separate stocks are indicated, but their value is ambiguous when they fail to detect stock structure (i.e., when they indicate panmixia). First of all, currently separate stocks might have shared an ancient genetic connection, the imprint of which has yet to fade. Also, even the transfer between stocks of a few individuals per generation is sufficient to maintain genetic homogeneity, yet such small transfers would have little effect on restoring overexploited populations. With this caveat in mind, management should regard the results of this study as representing the minimum number of stocks present. Further, the management plans should incorporate other rational concepts for delineating management units. For instance, despite their apparent panmixia, it would be reasonable to treat the Altantic longfin squid north versus south of Cape Hatteras separately, given that squid abundances and environmental factors are so clearly different between those areas. Similarly, the Atlantic and northeastern Gulf of Mexico arrow squid should probably be managed separately. Although there might be present-day gene flow between those two areas, the Florida Straits probably limit physical migrations to the point that a stock collapse in one area might not be restored in any reasonable time-frame from the other area. Of course, management options for arrow squid would change if future research should verify that North American waters are recolonized annually from more southern regions.

As long as southern U.S. squid stocks are not subject to targeted fisheries, there is probably little cause for concern regarding their viability. However, in the northeastern U.S., Brodziak (1998) noted that the long-term potential yield of longfin squid was recently cut in half (given revised biological data, particularly with regard to lifespan) and that a limited-entry system was necessary to protect the stock from overfishing. If targeted squid fisheries begin to develop south of Cape Hatteras, managers would be well-advised to proactively institute limited-entry controls on commercial fishing rather then react later to overcapitalization of these fisheries. Further, Hanlon (1998) raised concerns about fishing pressure on spawning

concentrations of *Loligo* species. Although he believes that moderate pressure will probably not adversely affect their mating systems, Hanlon cautioned that these systems should be better understood prior to allowing high levels of exploitation.

Future Studies: Based on the sequenced specimens, the RFLP analyses detected less than 50% of the variable nucleotide sites within each species of *Loligo* (Table 5). For both species, this resulted in sequence divergences within haplotypes that were often as large as those found between haplotypes. As such, these species might be more strongly structured than is apparent from the geographic distributions of the existing PCR-RFLP haplotypes. For instance, in longfin squid, sequences for the Gulf and Atlantic representatives of Haplotype B were separated by a single transition. In arrow squid, sequences of the eastern and western representatives of Haplotype A were separated by two transitions; further, all three representatives of Haplotype C had unique sequences. If these longfin and arrow squid sequences are truly representative of the haplotypes within their respective populations, then the existing haplotypes would represent composites of largely allopatric – and currently unrecognized – haplotypes. At a minimum, it would be worthwhile sequencing additional specimens of these three haplotypes to see if the patterns hold true. If so, less expensive methods could be used to verify the new haplotypes and their geographic distributions.

Regardless of the results of the proposed sequencing, digestions with additional restriction enzymes are likely to further subdivide the common PCR-RFLP mtDNA haplotypes. Nevertheless, unless it results in geographic clustering of the new haplotypes, such partitioning might actually reduce the signal of population structure through greater increases in the haplotype variance within subpopulations relative to the total population. Further, when studies use only a single marker, there is always the risk that the analysis has tracked the phylogeography of the gene rather than that of the organism. This problem can be avoided through comparisons of multiple and independent genetic markers. If such markers show

concordant phylogeography, then there is assurance that the analyses have tracked the phylogeography of the organism (Avise, 1996; Cunningham & Collins 1994; Hare, 1998). Genes within the mtDNA molecule are inherited as a single unit; therefore, they are not independent. As a result, rather than further subdividing the mtDNA marker or analyzing another mtDNA gene, it would be more profitable to use nuclear genetic markers. Suitable candidates such as microsatellites, introns, and ribosomal DNA are suggested in Appendix B.

Concluding Remarks: The above comments notwithstanding, the following can be concluded regarding the null hypotheses of this study:

- H_0-1: One of the original classifications (electrophoretic or morphometric) of the six putative hybrids found by Sánchez (1995) was incorrect, and the specimens were either longfin squid or arrow squid (see Appendix A: Hybrid Analysis).

  The null hypothesis is accepted. Although the original total protein electrophoretic data diagnosed the specimens as *L. pealei*, three other methods (allozymes, mtDNA PCR-RFLP's, and morphometric characters) clearly showed that the specimens were *L. plei*. Given the difficulties of interpreting data from the technique used by Sánchez (1995), his electrophoretic results are discounted.

- H_0-2: Neither longfin nor arrow squid is composed of cryptic species within the northern Gulf of Mexico and the western Atlantic Ocean.

  The null hypothesis is accepted. Within each species, nucleotide divergences among PCR-RFLP haplotypes (of the mitochondrial gene CO-I) were more than an order of magnitude lower than typically seen between species of loliginids.

- H_0-3: Gene flow within both species is consistent with a model of panmixia (i.e., Fst = 0 across the study area).

  The null hypothesis is rejected. Unlike for some pelagic fishes, F_{st} is not equal to zero for either longfin squid or for arrow squid across the northern Gulf of

Mexico and the northwestern Atlantic Ocean. Instead, both species consist of two populations characterized by strong genetic subdivision. At present, fishery management plans should follow a two-stock hypothesis for both species, although consideration should be given to further subdivision based on environmental factors and apparent abundances of each species within different regions.

- H_0-4: Population structure differing from panmixia is concordant with the classic Gulf of Mexico-Atlantic Ocean phylogeographic pattern that has been seen for other marine taxa in the region.

The null hypothesis is accepted for longfin squid, in which populations are separated by the Florida peninsula. In contrast, the null hypothesis is rejected for arrow squid, in which populations are separated at the Mississippi River into a northwestern Gulf population and a northeastern Gulf-Atlantic population.

The two phylogeographic patterns are probably determined by a combination of differences between the species in terms of their southern ranges and their life history characteristics. For instance, longfin squid are restricted to the continental shelf; thus, the high temperatures and strong eastward currents of the Florida Straits would limit gene flow to one direction (i.e., Gulf to Atlantic). In contrast, arrow squid are found throughout the Caribbean Sea, making it likely that the western and eastern populations are in genetic contact across the southern Gulf of Mexico. Although other explanations exist for the abrupt genetic break in this species, it seems most probable that the break results from annual recolonization of the northern Gulf of Mexico by squid from more southern populations. As squid immigrating from southern Mexico and from the eastern Caribbean moved toward the center of the northern Gulf, their advance would be impeded by the low salinities created by the Mississippi River. The lack of any effect by the river on the genetic structure of longfin squid is probably due to the habitat of that species being farther offshore than is true for arrow squid.

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APPENDIX A: HYBRID ANALYSIS

During his study of morphometric variation in longfin and arrow squid, Sánchez (1995) found six individuals that were scored as longfin squid by total protein electrophoresis and as arrow squid by morphometric analyses. This finding generated excitement because hybridization in cephalopods had not previously been documented (M. Vecchione, NMFS Systematics Laboratory, National Museum of Natural History, Washington, D.C., personal communication). These specimens were not discussed by Sánchez, but they were given to me for further analysis by allozyme electrophoresis. Although the high degree of allozyme monomorphism in cephalopods makes this technique problematic for population studies, it makes allozymes especially valuable for distinguishing species and for identifying hybrids.

Six specimens per species (longfin, arrow, and brief squid) and the six putative hybrids were surveyed for polymorphisms in a total of 18 enzyme systems (Table A-1) using electrophoretic buffers that had successfully distinguished *Loligo vulgaris* from *L. reynaudii* (Augustyn & Grant, 1988): Lithium hydroxide (pH 8.5/8.1); Tris-citrate (pH 6.0, 6.9, and 8.0); and, Tris-borate (pH 8.7). Soluble proteins were extracted from about 300 mg of frozen mantle tissue ground in 600 µl of cold Tris-20% Glycerol buffer (pH 8.0). The homogenates were briefly centrifuged at 4°C. Samples were then electrophoresed on horizontal 12% starch gels (Sigma Chemical Co.) and stained according to procedures outlined in May (1998).

The putative hybrids had been thawed multiple times by Sánchez (1995) and stored frozen for over two years; the other specimens had been thawed and refrozen once while on the survey vessel and then stored frozen for over a month. Thus, most of the allozymes analyzed for this project were too degraded to allow confident scoring. Nevertheless, probable interspecific polymorphisms were uncovered in 10 enzymes: *Eno*, *Got*, *Idh*, *Mdh*, *Me*, *Odh*, *Pep*, *Pgd*, *Pgi*, and *Pgm*. Intraspecific polymorphisms were not revealed by the limited sample size. Enzymes were inactive or severely degraded for *Hk*, *Ldh*, and *Xdh*; no activity was found

Table A-1. Pair-wise allozyme comparisons of three species of loliginid squids with five electrophoresis buffers (e.g., Augustyn & Grant, 1988). Partial degradation of the mantle tissue enzymes was common; thus, all results should be interpreted cautiously.

Frozen mantle tissue (~300 mg) ground in 600 µl of cold Tris-20% Glycerol buffer, pH 8.0.		Pair-wise comparisons of *Loligo pealei* [1]; *Loligo plei* [2]; and, *Lolliguncula brevis* [3]														
		1:2	1:3	2:3	1:2	1:3	2:3	1:2	1:3	2:3	1:2	1:3	2:3	1:2	1:3	2:3
Enzyme		LiOH pH 8.5/8.1[a]			TBE pH 8.7[b]			TC pH 6.0[c]			TC pH 6.9[d]			TC pH 8.0[e]		
Enolase	*Eno**	–	–	–	–	–	–	–	–	–	–	–	–	Y	Y	Y
Glutamate oxaloacetate transferase	*Got**	D[1]	Y[2]	D	Y	I[3]	I	D	Y	D	Y	Y	Y	–[4]	–	–
Isocitrate dehydrogenase	*Idh*	–	–	–	I	I	I	–	–	–	Y	P[5]	Y	N[6]	N	N
Malate dehydrogenase	*Mdh*	N	Y	Y	N	Y	Y	–	–	–	N	N	N	N	D	D
Malic enzyme	*Me*	Y	Y	N	I	Y	I	P	P	P	P	P	P	N	N	N
Octopine dehydrogenase	*Odh*	Y	Y	Y	Y	Y	Y	P	P	P	I	P	I	I	I	I
Peptidase (L-leucine-L-alanine)	*Pep**	–	–	–	–	–	–	–	–	–	Y	Y	Y	–	–	–
Phosphogluconate dehydrenase	*Pgd*	–	–	–	P	Y	Y	–	–	–	Y	P	Y	–	–	–
Phosphoglucose isomerase	*Pgi*	Y	Y	N	D	D	D	D	D	D	Y	D	D	–	–	–
Phosophoglucomutase	*Pgm**	Y	Y	N	Y	Y	P	Y	N	N	Y	Y	P	–	–	–
Fumerase	*Fum*	I	I	I	–	–	–	I	I	I	–	–	–	–	–	–
Glyceraldehydephosphate dehydro.	*Gap*	I	I	I	–	–	–	–	–	–	I	I	I	–	–	–
Hexokinase	*Hk*	–	–	–	D	I	I	–	–	–	I	D	I	–	–	–
Lactate dehydrogenase	*Ldh*	I	I	I	–	–	–	I	I	I	–	–	–	D	D	D
Peptidase (L-leucine)	*Lap*	I	I	I	–	–	–	I	I	I	–	–	–	–	–	–
Mannose-phosphate isomerase	*Mpi*	I	I	I	–	–	–	I	I	I	–	–	–	–	–	–
Superoxide dismutase	*Sod*	I	I	I	–	–	–	I	I	I	–	–	–	–	–	–
Xanthine dehydrogenase	*Xdh*	I	I	I	–	–	–	I	I	I	–	–	–	D	D	D

[a] 2 squid/species; [b] 4 squid/species; [c] 2 squid/species; [d] 4 squid/species; [e] 1 squid/species.

[1] (D) severe degradation of enzyme in one or both species; [2] (Y) allele migration difference between the two species compared;

[3] (I) not detectable in one or both species; [4] (–) not tested with buffer; [5] (P) possible allele migration difference; [6] (N) no migration difference.

* Active enzyme for putative hybrids; results matched the banding pattern of *L. plei* (arrow squid) in each case.

for *Fum*, *Gap*, *Lap*, *Mpi*, and *Sod*. Given the degree of enzyme degradation, the results are reported only in qualitative terms. Even as qualitative measures, the results in Table A-1 should be interpreted cautiously and used solely as a guide to future studies of these species.

Only four enzymes remained active in the six putative hybrids: *Eno*, *Got*, *Pep*, and *Pgm*. In each case, the allozyme patterns clearly matched those of arrow squid. As further verification, I amplified the CO-I gene by PCR from these specimens and digested the PCR products as described for the two *Loligo* species. The mtDNA digest patterns also matched those of arrow squid. Thus, with the exception of the total protein gels, all data (allozymes, morphometrics, and mtDNA) indicated that the putative hybrids were *L. plei*. Given the difficulty of interpreting banding patterns in the technique used by Sánchez (1995), these specimens appear to have been simply misdiagnosed as *L. pealei* by the total protein gels.

After determining that the specimens in question were not hybrids of *L. pealei* and *L. plei*, the question was whether to continue this approach for the population analysis. Allozymes can rapidly provide large amounts of genetic data, and they have been used with moderate success in cephalopods for intraspecific studies (e.g., Brierley et al., 1993; Brierley et al., 1995; Garthwaite et al., 1989; Yeatman & Benzie, 1994). However, cephalopod allozymes generally show a low degree of allelic polymorphism. Further, even while frozen, some of the typically polymorphic enzymes degrade within a year or sometimes even more rapidly (e.g., Glutamate oxaloacetate transferase degrades in < 1 month) (Carvalho & Loney, 1989). It is possible that experimentation with different grinding buffer recipes would have resolved much of the difficulty with enzyme degradation seen in this study – at least with the specimens collected after 1993. However, the prospects of this approach were not promising for defining intraspecific population structure in longfin and arrow squid; hence, this research was terminated in favor of the PCR-RFLP approach with mitochondrial DNA.

APPENDIX B: NUCLEAR DNA PRIMERS

Concordance in population genetic structure is not always the case for mtDNA and nuclear DNA (Cunningham & Collins 1994). Therefore, to continue this research, nuclear markers should be developed. Given the success of Shaw et al. (1999) at elucidating subtle population structure in *Loligo forbesi*, microsatellite analysis might be the most powerful genetic technique currently available for providing a nuclear marker. Although no microsatellite PCR primers have been published for the longfin, arrow, or brief squid, a microsatellite primer system is being developed for longfin squid (R. Hanlon, Marine Biological Laboratory, Woods Hole, Massachusetts, personal communication). It is doubtful that the system could be transferred to the other two species; hence, microsatellites might have to be developed for each species. In fact, if cryptic speciation exists in the Caribbean or South American loliginid populations, more than three microsatellite systems might be required.

Primers for other types of nuclear markers are more likely to be applicable to all three species. Currently, two good candidates appear to be an intron from the neuronal filament (NF70) protein gene and the ribosomal gene ITS-1 (internal transcribed spacer region between the 18S and 28S nuclear rDNA coding regions). The NF70 gene was chosen because the locations of the introns within this gene are highly conserved across phylogenetically divergent taxa (Way et al., 1992); consequently, primers could be designed based on cDNA sequences found in databases such as GenBank (Benson et al., 1999). The third intron was targeted because its size in other species was about 500 bp, which would be amenable to sequencing. For ITS-1, interspecific sequence divergences ranged from 2% to 12% in 11 species of sepiolid squids (Nishiguchi et al., 1998). No differences were found between separate clones of ITS-1 from the same individual ($n = 2$ or 3 clones from one squid per species); these results were consistent with other studies which also found that ITS-1, despite its high copy number, could be directly sequenced (Caporale et al., 1997). Finally, although the ITS-1 intraspecific

divergences were low for sepiolid squids (< 0.1%; $n = 2$ to 3 squid per species), the gene still might be sufficiently variable for phylogeographic resolution.

Several months were spent developing primers for these nuclear markers (Table B-1). Two sets of primers were used for ITS-1, with one set designed for algae (Goff et al., 1994) and the other set for the mussel, *Mytilus* (Heath et al., 1995). For the NF70 intron, I designed three forward primers and two reverse primers based on sequences from longfin squid (*Loligo pealei*; GenBank accession number M64718) and a snail (*Helix aspera*; accession number X86347); sequences were aligned in ClustalW v. 1.7 (Thompson et al., 1994). The most inclusive NF70 primers were 59 bp and 123 bp outside the intron (which is located between positions 834 and 835 in the squid cDNA sequence) so as to provide an identifying portion of the exon in the PCR product. To help prevent amplification of genes from the other five classes of the intermediate-filament (IF) gene family (NF70 is classified as type IV), separate alignments of the squid NF70 sequence were done with two IF genes: (1) the IF-protein gene

Table B-1. PCR primers tested in *Loligo pealei*, *L. plei*, *Lolliguncula brevis*, and *Illex coindetti*. The two ITS-18s primers are anchored in the same location on the gene. The numbers in the NF70 designations refer to the 5' nucleotide position in the NF70 cDNA sequence (GenBank accession number M64718).

Gene	Primer	Primer Sequence	Product Size
CO-I[1]	H-COI	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'	709 bp
	L-COI	5'-GGTCAACAAATCATAAAGATATTGG-3'	
ITS[2]	ITS-18s	5'-GGGATCCTTTCCGTAGGTGAACCTGC-3'	~1,200 bp
	ITS-28s	5'-GGGATCCATATGCTTAAGTTCAGCGGGT-3'	
ITS[3]	ITS-18s	5'-GTTTCCGTAGGTGAACCTG-3'	~800 bp
	ITS-28s	5'-CTCGTCTGATCTGAGGTCG-3'	
NF70[4]	NF70-756F	5'-TATCAATCATTTGGACGCTG-3'	~1,300 bp
	NF70-782F	5'-GCAGACAAACACTTGAGGAAG-3'	
	NF70-791F	5'-CACTTGAGGAAGAACTTGAATT-3'	
	NF70-946R	5'-CGTCATACTCTTGTTGGATGTC-3'	
	NF70-977R	5'-TCAATATCGCCTCTCATTTG-3'	

[1]Folmer et al. 1994; [2]Goff et al. 1994; [3]Heath et al. 1995; [4]Designed by S. Herke.

from another squid (*Ommastrephes sloani*; accession number L10112); and, (2) the omega-crystaline IF gene from an octopus (*Octopus dofleini*; accession number L10113).

PCR was done as described for CO-I except that, following the hot start, the PCR cycle for the nuclear markers was 35 – 44 cycles of 95°C (30 s); 58°C (30 s); and 72°C (60 s). Initially, high quality, robust amplifications were achieved for ITS-1 and the third NF70 intron (with all combinations of forward and reverse primers) from fresh salt-extractions of squid DNA. Subsequent amplifications with the NF70 primers using the same DNA extractions gradually decreased in quality and eventually failed altogether, whereas these extractions continued to generate strong amplifications with primers for both CO-I and ITS-1. CO-I and ITS-1 are both multicopy genes while NF70 is supposedly a single-copy gene. Thus, it appeared that the extracted template DNA was degrading. I then switched from the salt-extraction to a phenol-chloroform DNA extraction (Appendix D). The new DNA extraction procedure provided high quality DNA and the NF70 amplifications were successful once again. Further, when used as template, the undiluted and non-purified NF70 PCR product generated robust amplifications even with the same primer pair as in the initial reaction.

Electrophoresis of the PCR products was done on 2% agarose gels, so all sizes are approximate. The Goff ITS-1 primers generated a 1,200 bp PCR product; the Heath primers generated an 800 bp PCR product. There was a visible size difference between genera in the NF70 intron (~1,300 bp), with a larger intron in *Loligo pealei* and *L. plei* as compared to *Lolliguncula brevis*. A weak amplification was obtained one time from *Illex coindetti*, but that species was tested only with DNA prepared by salt-extraction. Assuming that the PCR product from *Illex* was not the result of contamination, the NF70 intron is much smaller in *Illex*. A partial sequence of the NF70 PCR product showed 100% identity with the 123 bp of the cDNA gene sequence (Way et al., 1992) located between the NF70-977R primer and the third intron; the ITS-1 products were not sequenced.

None of the PCR products was rigorously tested for intraspecific nucleotide variation (either by sequence or RFLP analyses). However, given the size variations seen in the third NF70 intron for the different genera, it seems likely that this intron will be useful for population-level genetic studies in squid. Characterization of the PCR products ceased because of DNA contamination problems that took several months to resolve. The standard pipette tips and autoclaved, double-distilled water initially used throughout the PCR process proved to be intermittent sources of contaminating DNA. Thus, they were replaced with Areoseal filter pipette tips (USA Scientific Plastics) and HPLC-grade water (Mallinckrodt) for both DNA extraction and amplification. Further, I began using PCR tubes that were certified as DNA-free. Nevertheless, the nuclear primer stocks themselves had become contaminated by then and these markers were not pursued further due to time constraints.

Determining the nucleotide sequences for PCR products from all specimens might have greater potential for detecting population subdivision than do the methods used in this study. However, cost concerns would typically require that sample sizes be significantly reduced. To avoid this trade-off, Single Strand Conformational Polymorphism (SSCP) has been used in other research to determine non-identity of PCR products by even a single base pair (Aguade et al., 1994). Thus, under the right circumstances, initial screening with SSCP can reduce the amount of sequencing required. Nevertheless, while SSCP might be useful for analysis of the ITS-1 gene and the NF70 gene, the majority of intraspecific PCR products for CO-I are likely to differ by at least 1 bp. In that case, substantial reductions in sequencing would not be realized by SSCP. Thus, at least initially, the best approach might be to continue with the methods used in this study (i.e., PCR-RFLP and limited sequencing). Once preliminary data have been obtained from the more southern populations of *Loligo*, as well as from *Lolliguncula brevis*, more sophisticated techniques can be applied to increase phylogeographic resolution.

APPENDIX C: SUMMARY DATA

The following tables contain all of the summary data for the specimens of longfin squid (*L. pealei*) and arrow squid (*L. plei*). The genetic data are based on restriction digests of a 709-bp fragment of a mtDNA gene – cytochrome *c* oxidase, subunit I (CO-I). Mantle lengths were measured to the nearest 5 mm on frozen specimens. Latitudes and longitudes for the squid capture locations were rounded to the nearest 0.5 degrees. Codes for restriction digests are interpreted as follows: 1st number is the designation for specific pattern of bands in a gel; 2nd number is the total restriction sites for that enzyme (e.g., "31" represents "Banding pattern 3 with 1 restriction site". An "nd" indicates that the digest was not done; with two exceptions, these digests were not actually necessary to diagnose the haplotypes of affected individuals. Codes for survey cruises are described in Table C-1.

Table C-1. Letter and date codes (used in Tables C-2 and 3) for the survey cruises from which samples of *L. pealei* (longfin) and *L. plei* (arrow squid) were obtained.

Code	Source of Squid Samples
A	R/V *Albatross* IV, (NOAA) NMFS, NE Fisheries Science Center, Woods Hole, MA.
B	Barans, Mr. C., South Carolina Department of Natural Resources.
C	R/V *Chapman*, (NOAA) NMFS, SE Fisheries Science Center, Pascagoula, MS.
D	Gregory, Mr. D., Sea Grant marine extension agent, Key West, FL.
R	R/V *Oregon* II, (NOAA) NMFS, SE Fisheries Science Center, Pascagoula, MS.
S	Sánchez samples from R/V *Oregon* II.

Code	Dates of the Survey Cruise
07/93	19 June 1993 – 21 July 1993.
07/95	17 June 1995 – 19 July 1995.
11/95	10 October 1995 – 17 November 1995.
02/96	5 – 29 February 1996.
04/96	4 March – 29 April 1996.
10/96	9 September – 31 October 1996.
05/97	14 April – 9 May 1997.
01/97	January 1997 (multiple trips by commercial shrimpers, Dry Tortugas Pink Shrimp).

Table C-2. *Loligo pealei* summary data: ID – squid identification number; Hap – mtDNA CO-I PCR-RFLP haplotype; Size – mantle length (nearest 5 mm); Unit – sample unit as described in Figures 1 and 2; °N – latitude; °W – longitude; Sta – Station in original cruise data; Cruise – See Table C-1. Restriction enzymes were *Bst*NI (□), *Hin*fI (▲), *Msp*I (○), and *Hae*III (■). See text of Appendix C (or Figure 4) for interpreting restriction data. Bold-faced entries highlight Haplotypes B-M and "non-Haplotype A" restriction patterns.

ID	Hap	Size	Unit	°N	°W	Sta	Cruise	□	▲	○	■
A001	A	290	10	42.5	66.5	286	A-04/96	11	10	12	13
A002	A	190	10	42.5	66.5	286	A-04/96	11	10	12	13
A003	A	210	10	42.5	66.5	286	A-04/96	11	10	12	13
A004	A	280	10	43.0	67.0	303	A-04/96	11	10	12	13
A005	A	190	10	40.5	68.0	244	A-04/96	11	10	12	13
A006	**C**[1]	170	10	40.5	68.0	244	A-04/96	11	10	nd	**22**
A007	A	130	10	40.5	68.0	244	A-04/96	11	10	12	13
A008	A	320	9	39.0	73.5	001	A-04/96	11	10	12	13
A009	A	230	9	39.0	73.5	001	A-04/96	11	10	12	13
A010	A	270	8	38.0	74.0	009	A-04/96	11	10	12	13
A011	A	200	8	38.0	74.0	009	A-04/96	11	10	12	13
A012	A	210	8	38.0	74.0	009	A-04/96	11	10	12	13
A013	A	125	8	38.5	73.5	002	A-04/96	11	10	12	13
A014	A	165	8	38.5	73.5	002	A-04/96	11	10	12	13
A015	A	230	8	38.5	73.5	002	A-04/96	11	10	12	13
A016	A	190	8	38.0	74.0	005	A-04/96	11	10	12	13
A017	A	175	8	38.0	74.0	005	A-04/96	11	10	12	13
A018	A	175	8	38.0	74.0	005	A-04/96	11	10	12	13
A019	A	225	8	38.5	73.5	003	A-04/96	11	10	12	13
A020	A	260	10	40.5	68.0	130	A-02/96	11	10	12	13
A021	**C**	180	10	40.5	68.0	131	A-02/96	11	10	**21**	**22**
A022	A	220	10	40.5	67.5	134	A-02/96	11	10	12	13
A023	A	220	10	40.5	67.5	134	A-02/96	11	10	12	13
A024	A	220	10	40.5	67.0	135	A-02/96	11	10	12	13
A025	**B**	190	10	40.5	67.0	135	A-02/96	11	**21**	12	13
A026	**B**	140	8	37.0	74.5	027	A-04/96	11	**21**	12	13
A027	A	190	8	37.0	74.5	027	A-04/96	11	10	12	13
A028	A	150	8	37.0	74.5	027	A-04/96	11	10	12	13
A029	A	120	8	37.0	74.5	027	A-04/96	11	10	12	13
A030	A	150	8	37.0	74.5	027	A-04/96	11	10	12	13
A031	**E**	160	7	35.5	75.0	026	A-10/96	**33**	10	12	13
A032	A	300	7	35.5	75.0	026	A-10/96	11	10	12	13
A034	A	290	7	35.5	75.5	037	A-10/96	11	10	12	13
A036	A	345	7	36.0	75.5	038	A-10/96	11	10	12	13
A037	A	185	7	36.0	75.5	039	A-10/96	11	10	12	13
A038	A	135	7	36.0	75.5	039	A-10/96	11	10	12	13
A039	A	190	7	36.5	75.5	044	A-10/96	11	10	12	13
A040	A	460	7	36.5	75.5	044	A-10/96	11	10	12	13
A041	A	260	7	36.5	75.5	045	A-10/96	11	10	12	13
A042	A	130	7	36.5	75.5	045	A-10/96	11	10	12	13
A043	**C**	145	7	36.5	76.0	046	A-10/96	11	10	**21**	**22**
A044	A	215	7	36.5	76.0	046	A-10/96	11	10	12	13
A045	**C**	150	8	37.0	75.5	047	A-10/96	11	10	**21**	**22**
A046	**B**	240	8	37.0	75.5	047	A-10/96	11	**21**	12	13
A047	A	130	7	37.0	76.0	050	A-10/96	11	10	12	13
A048	**B**	220	7	37.0	76.0	050	A-10/96	11	**21**	12	13
A049	A	165	8	37.5	75.5	051	A-10/96	11	10	12	13
A050	A	170	8	37.5	75.5	051	A-10/96	11	10	12	13

(Table C-2 continued)

ID	Hap	Size	Unit	°N	°W	Sta	Cruise	□	▲	○	■
A051	**C**	145	10	42.0	70.0	221	A-10/96	11	10	**21**	**22**
A052	**C**	100	10	42.0	70.0	221	A-10/96	11	10	**21**	**22**
A053	A	80	10	40.5	68.0	244	A-04/96	11	10	12	13
A054	A	90	10	40.5	68.0	244	A-04/96	11	10	12	13
A055	**E**	160	9	40.0	70.5	113	A-02/96	**33**	10	12	13
A056	A	140	9	40.0	70.5	113	A-02/96	11	10	12	13
A057	A	150	10	40.0	69.5	115	A-02/96	11	10	12	13
A058	A	160	10	40.0	69.5	115	A-02/96	11	10	12	13
A059	A	260	10	40.0	69.5	116	A-02/96	11	10	12	13
A060	A	200	10	40.0	69.5	116	A-02/96	11	10	12	13
A061	A	300	10	40.0	69.5	117	A-02/96	11	10	12	13
A062	**C**	150	10	40.0	69.5	117	A-02/96	11	10	**21**	22
A063	**L**	70	10	40.0	69.0	118	A-02/96	**42**	10	12	13
A064	**C**	270	10	40.0	69.0	118	A-02/96	11	10	**21**	**22**
A065	**E**	190	10	40.0	69.5	115	A-02/96	**33**	10	12	13
A066	A	250	10	40.0	69.5	116	A-02/96	11	10	12	13
A067	A	180	10	40.0	69.5	117	A-02/96	11	10	12	13
A068	**E**	210	9	40.0	70.5	113	A-02/96	**33**	10	12	13
A069	A	170	10	40.5	67.5	134	A-02/96	11	10	12	13
A070	**C**	200	10	40.5	67.5	134	A-02/96	11	10	**21**	**22**
A071	**E**	180	10	40.0	69.0	118	A-02/96	**33**	10	12	13
A072	A	200	10	40.0	69.0	118	A-02/96	11	10	12	13
A073	**D**	200	10	40.5	67.0	135	A-02/96	**22**	10	nd	13
A074	A	150	10	40.5	67.0	135	A-02/96	11	10	12	13
A075	**D**	200	10	40.5	67.0	135	A-02/96	**22**	10	nd	13
A076	A	200	9	39.5	72.5	006	A-02/96	11	10	12	13
A077	A	160	9	39.5	72.5	006	A-02/96	11	10	12	13
A078	**C**	140	9	39.5	72.5	011	A-02/96	11	10	**21**	**22**
A079	**D**	210	9	39.5	72.5	011	A-02/96	**22**	10	nd	13
A080	**H**	190	9	39.0	73.0	015	A-02/96	11	**21**	12	**22**
A081	A	280	9	39.0	73.0	015	A-02/96	11	10	12	13
A082	A	220	9	39.0	73.0	016	A-02/96	11	10	12	13
A083	A	105	9	39.0	73.0	016	A-02/96	11	10	12	13
A084	**C**	240	9	40.0	72.0	097	A-02/96	11	10	**21**	**22**
A085	A	150	9	40.0	72.0	097	A-02/96	11	10	12	13
A086	A	220	9	40.0	72.0	098	A-02/96	11	10	12	13
A087	A	155	9	40.0	72.0	098	A-02/96	11	10	12	13
A088	**B**	210	9	40.0	71.5	099	A-02/96	11	**21**	12	13
A089	A	145	9	40.0	71.5	099	A-02/96	11	10	12	13
A090	A	265	9	40.0	70.5	113	A-02/96	11	10	12	13
A091	A	150	9	40.0	70.5	113	A-02/96	11	10	12	13
A092	**K**	280	10	40.0	69.5	116	A-02/96	**22**	**21**	12	13
A093	A	170	10	40.0	69.5	116	A-02/96	11	10	12	13
A094	A	185	10	40.0	69.5	117	A-02/96	11	10	12	13
A095	**F**	155	10	40.0	69.5	117	A-02/96	11	10	12	**22**
A096	**C**	130	10	40.0	69.0	118	A-02/96	11	10	**21**	**22**
A097	**E**	150	7	36.5	75.0	018	A-10/96	**33**	10	12	13
A098	A	190	7	36.5	75.0	018	A-10/96	11	10	12	13
A099	A	340	7	35.5	75.0	026	A-10/96	11	10	12	13
A100	A	200	7	35.5	75.5	037	A-10/96	11	10	12	13
A101	A	130	7	36.0	75.5	038	A-10/96	11	10	12	13

(Table C-2 continued)

ID	Hap	Size	Unit	°N	°W	Sta	Cruise	□	▲	○	■
A103	A	200	7	36.5	75.5	044	A-10/96	11	10	12	13
A104	A	100	7	36.5	75.5	045	A-10/96	11	10	12	13
A106	A	170	8	37.0	75.5	047	A-10/96	11	10	12	13
A107	A	140	7	37.0	76.0	050	A-10/96	11	10	12	13
A108	A	160	8	37.5	75.5	051	A-10/96	11	10	12	13
A109	A	230	8	38.0	74.5	008	A-10/96	11	10	12	13
A110	A	170	8	38.0	74.5	008	A-10/96	11	10	12	13
A111	A	160	8	38.0	74.0	009	A-10/96	11	10	12	13
A112	A	220	8	38.0	74.0	009	A-10/96	11	10	12	13
A113	**E**	180	8	37.5	75.5	060	A-10/96	**33**	10	12	13
A114	A	150	8	37.5	75.5	060	A-10/96	11	10	12	13
A115	A	110	8	38.0	75.0	065	A-10/96	11	10	12	13
A116	**B**	150	8	38.0	75.0	065	A-10/96	11	**21**	12	13
A117	A	230	8	38.5	74.5	071	A-10/96	11	10	12	13
A118	**F**	200	8	38.5	74.5	071	A-10/96	11	10	12	**22**
A119	A	160	8	38.5	74.5	073	A-10/96	11	10	12	13
A120	A	180	8	38.5	74.5	073	A-10/96	11	10	12	13
A121	**C**	220	9	39.0	74.5	074	A-10/96	11	10	**21**	**22**
A122	A	160	9	39.0	74.5	074	A-10/96	11	10	12	13
A123	**H**	170	9	39.0	74.5	076	A-10/96	11	**21**	12	**22**
A124	A	120	9	39.0	74.5	076	A-10/96	11	10	12	13
A125	A	160	9	39.0	74.0	086	A-10/96	11	10	12	13
A126	A	170	9	39.0	74.0	086	A-10/96	11	10	12	13
A127	**F**	380	9	39.5	73.5	095	A-10/96	11	10	12	**22**
A128	A	160	9	39.5	73.5	095	A-10/96	11	10	12	13
A129	A	120	9	40.0	73.5	104	A-10/96	11	10	12	13
A130	A	170	9	40.0	73.5	104	A-10/96	11	10	12	13
A131	A	340	9	40.0	73.5	105	A-10/96	11	10	12	13
A132	A	120	9	40.0	73.5	105	A-10/96	11	10	12	13
A133	A	150	9	40.0	73.5	106	A-10/96	11	10	12	13
A134	**B**	170	9	40.0	73.5	106	A-10/96	11	**21**	12	13
A135	A	170	9	40.0	73.5	106	A-10/96	11	10	12	13
A136	A	110	10	41.0	69.5	170	A-10/96	11	10	12	13
A137	**C**	110	10	41.0	69.5	170	A-10/96	11	10	**21**	**22**
B035	**G**	145	6	31.0	81.0	069	B-05/97	11	10	12	**32**
B050	A	100	6	31.5	81.0	064	B-05/97	11	10	12	13
B059	A	85	6	33.0	79.5	012	B-05/97	11	10	12	13
B060	A	80	6	33.0	79.5	012	B-05/97	11	10	12	13
B062	A	80	6	33.0	79.5	012	B-05/97	11	10	12	13
B063	**B**	95	6	33.0	79.5	012	B-05/97	11	**21**	12	13
B207	A	115	6	34.0	78.0	159	B-05/97	11	10	12	13
B208	A	120	6	34.0	78.0	159	B-05/97	11	10	12	13
B209	A	105	6	34.0	78.0	159	B-05/97	11	10	12	13
B210	A	120	6	34.0	78.0	159	B-05/97	11	10	12	13
B211	A	185	6	34.0	78.0	159	B-05/97	11	10	12	13
B212	A	140	6	34.0	78.0	159	B-05/97	11	10	12	13
B213	**C**	115	6	34.0	78.0	159	B-05/97	11	10	**21**	**22**
B214	A	130	6	34.0	78.0	159	B-05/97	11	10	12	13
B215	A	125	6	34.0	78.0	159	B-05/97	11	10	12	13
B216	A	120	7	35.0	76.0	145	B-05/97	11	10	12	13
B217	A	115	6	33.0	79.5	012	B-05/97	11	10	12	13
B218	A	140	7	34.5	77.0	155	B-05/97	11	10	12	13
B219	A	95	7	34.5	77.0	155	B-05/97	11	10	12	13

(Table C-2 continued)

ID	Hap	Size	Unit	°N	°W	Sta	Cruise	□	▲	○	■
B220	A	120	7	34.5	77.0	155	B-05/97	11	10	12	13
B221	A	110	7	35.0	76.0	150	B-05/97	11	10	12	13
B222	A	90	7	35.0	76.0	150	B-05/97	11	10	12	13
B223	**F**	105	7	35.0	76.0	150	B-05/97	11	10	12	**22**
B224	**E**	155	6	31.0	81.0	071	B-05/97	**33**	10	12	13
B225	A	140	6	32.5	80.0	049	B-05/97	11	10	12	13
B226	A	110	7	35.0	76.0	149	B-05/97	11	10	12	13
B227	**E**	160	7	35.0	76.0	149	B-05/97	**33**	10	12	13
B228	A	120	6	34.0	78.0	133	B-05/97	11	10	12	13
B229	A	130	6	34.0	78.0	133	B-05/97	11	10	12	13
B230	A	50	7	35.0	76.5	144	B-05/97	11	10	12	13
B231	A	55	7	35.0	76.5	144	B-05/97	11	10	12	13
B232	A	135	7	35.0	76.5	144	B-05/97	11	10	12	13
B233	**B**	180	6	32.5	80.0	054	B-05/97	11	**21**	12	13
B234	**C**	135	6	34.0	78.0	160	B-05/97	11	10	**21**	**22**
B235	A	155	6	34.0	78.0	160	B-05/97	11	10	12	13
B236	A	170	6	34.0	78.0	160	B-05/97	11	10	12	13
B237	A	130	6	34.0	78.0	160	B-05/97	11	10	12	13
B238	**C**	185	6	34.0	78.0	160	B-05/97	11	10	**21**	**22**
B239	A	120	7	35.0	76.5	143	B-05/97	11	10	12	13
B240	A	105	7	35.0	76.5	143	B-05/97	11	10	12	13
B241	A	70	7	35.0	76.5	143	B-05/97	11	10	12	13
B242	A	90	7	35.0	76.5	143	B-05/97	11	10	12	13
B243	**C**	155	6	34.0	78.0	162	B-05/97	11	10	**21**	**22**
B244	**C**	115	6	34.0	78.0	162	B-05/97	11	10	**21**	**22**
B245	**A**	130	6	33.5	78.0	139	B-05/97	11	10	12	13
B246	A	130	7	35.0	76.0	146	B-05/97	11	10	12	13
B247	**B**	145	6	34.0	78.0	164	B-05/97	11	**21**	12	13
B248	A	135	6	34.0	78.0	164	B-05/97	11	10	12	13
B249	**B**	120	6	33.5	78.0	140	B-05/97	11	**21**	12	13
B250	A	110	6	34.0	78.5	043	B-05/97	11	10	12	13
B251	A	155	6	34.0	78.5	043	B-05/97	11	10	12	13
B252	A	115	6	34.0	78.5	043	B-05/97	11	10	12	13
B253	A	140	7	34.0	78.5	043	B-05/97	11	10	12	13
B254	A	120	6	34.5	77.5	157	B-05/97	11	10	12	13
B255	A	95	6	34.5	77.5	157	B-05/97	11	10	12	13
B256	**C**	75	6	34.5	77.5	157	B-05/97	11	10	**21**	**22**
B257	A	100	6	34.5	77.5	157	B-05/97	11	10	12	13
C001	A	280	1	27.0	96.5	005	C-11/95	11	10	12	13
C002	**B**	150	2	28.0	93.5	031	C-11/95	11	**21**	12	13
C003	A	170	2	29.0	94.0	026	C-11/95	11	10	12	13
C004	A	140	2	29.0	94.0	026	C-11/95	11	10	12	13
C005	A	170	2	27.0	93.5	031	C-11/95	11	10	12	13
C006	A	220	2	28.0	93.0	038	C-11/95	11	10	12	13
C007	**I**	130	2	28.0	93.0	038	C-11/95	11	**21**	12	**42**
C008	**D**	120	2	28.0	93.0	038	C-11/95	**22**	10	12	13
C009	A	120	2	28.5	94.0	023	C-11/95	11	10	12	13
C010	A	170	4	30.0	87.0	080	C-11/95	11	10	12	13
C011	A	150	4	30.0	87.0	080	C-11/95	11	10	12	13
C012	**D**	260	4	30.0	88.0	070	C-11/95	**22**	10	12	13
C013	**B**	200	5	29.0	84.0	102	C-11/95	11	**21**	12	13
C014	A	150	4	30.0	86.5	084	C-11/95	11	10	12	13
C015	**B**	140	4	30.0	86.5	084	C-11/95	11	**21**	12	13

(Table C-2 continued)

ID	Hap	Size	Unit	°N	°W	Sta	Cruise	□	▲	O	■
C016	A	160	4	30.0	86.5	084	C-11/95	11	10	12	13
C017	A	130	4	30.0	88.0	071	C-11/95	11	10	12	13
C018	**B**	140	4	30.0	88.0	071	C-11/95	11	**21**	12	13
C019	A	140	4	30.0	86.5	090	C-11/95	11	10	12	13
C020	A	160	4	30.0	86.5	090	C-11/95	11	10	12	13
C021	A	170	5	29.0	86.0	095	C-11/95	11	10	12	13
C022	A	140	5	29.0	86.0	095	C-11/95	11	10	12	13
C023	A	260	5	28.5	85.0	107	C-11/95	11	10	12	13
C024	**D**	340	5	28.5	85.0	107	C-11/95	**22**	10	12	13
C025	**B**	350	5	28.5	85.0	107	C-11/95	11	**21**	12	13
C026	A	130	4	30.0	86.5	085	C-11/95	11	10	12	13
C027	**B**	130	4	30.0	86.5	085	C-11/95	11	**21**	12	13
C028	**B**	160	4	30.0	86.5	086	C-11/95	11	**21**	12	13
C029	**B**	160	4	30.0	86.5	086	C-11/95	11	**21**	12	13
C030	**B**	170	4	30.0	86.5	088	C-11/95	11	**21**	12	13
C031	A	120	4	30.0	86.5	088	C-11/95	11	10	12	13
C032	A	230	4	30.0	86.5	089	C-11/95	11	10	12	13
C033	**B**	180	4	30.0	86.5	089	C-11/95	11	**21**	12	13
C034	A	110	5	29.0	86.0	093	C-11/95	11	10	12	13
C035	A	150	5	29.0	86.0	093	C-11/95	11	10	12	13
C036	A	130	5	29.0	86.0	094	C-11/95	11	10	12	13
C037	**B**	80	5	29.0	86.0	094	C-11/95	11	**21**	12	13
C038	A	140	5	28.5	85.5	099	C-11/95	11	10	nd	13
C039	**J**	130	5	28.5	85.5	099	C-11/95	11	**21**	12	**32**
C040	**B**	130	5	28.5	85.5	100	C-11/95	11	**21**	12	13
C041	A	150	5	28.5	85.5	100	C-11/95	11	10	12	13
C042	A	240	5	28.5	85.0	106	C-11/95	11	10	12	13
C043	A	190	5	28.5	85.0	107	C-11/95	11	10	12	13
C044	A	210	5	28.5	85.0	107	C-11/95	11	10	12	13
C045	A	50	5	28.5	85.0	108	C-11/95	11	10	12	13
C046	**B**	80	5	28.5	85.0	108	C-11/95	11	**21**	12	13
C047	A	170	4	30.0	86.5	083	C-11/95	11	10	12	13
C048	A	210	4	30.0	86.5	083	C-11/95	11	10	12	13
C049	A	340	4	30.0	86.5	083	C-11/95	11	10	12	13
C050	**M**	180	4	30.0	86.5	083	C-11/95	11	10	**31**	13
C051	A	175	4	30.0	86.5	084	C-11/95	11	10	12	13
C052	A	130	4	30.0	86.5	085	C-11/95	11	10	12	13
C053	A	150	4	30.0	86.5	086	C-11/95	11	10	12	nd
C054	A	150	4	30.0	86.5	086	C-11/95	11	10	12	13
C055	**B**	160	4	30.0	86.5	086	C-11/95	11	**21**	12	13
C056	A	100	5	29.0	86.0	093	C-11/95	11	10	12	13
C057	A	265	5	29.0	86.0	095	C-11/95	11	10	12	13
C058	A	215	5	29.0	86.0	095	C-11/95	11	10	12	13
C059	**B**	200	5	29.0	86.0	095	C-11/95	11	**21**	12	13
C060	A	55	5	28.5	85.5	099	C-11/95	11	10	12	13
C061	A	65	5	28.5	85.5	099	C-11/95	11	10	12	13
C062	A	35	5	28.5	85.5	099	C-11/95	11	10	12	13
C063	A	110	5	28.5	85.5	100	C-11/95	11	10	12	13
C064	A	65	5	28.5	85.5	100	C-11/95	11	10	12	13
C065	**B**	125	5	28.5	85.5	100	C-11/95	11	**21**	12	13
C066	**B**	200	5	28.5	85.0	105	C-11/95	11	**21**	12	13
C067	**B**	145	5	28.5	85.0	105	C-11/95	11	**21**	12	13
C068	**B**	125	5	28.5	85.0	105	C-11/95	11	**21**	12	13

(Table C-2 continued)

ID	Hap	Size	Unit	°N	°W	Sta	Cruise	□	▲	O	■
C069	A	150	5	28.5	85.0	105	C-11/95	11	10	12	13
C070	**F**[2]	275	5	28.5	85.0	106	C-11/95	11	10	nd	**22**
C071	**B**	85	5	28.5	85.0	106	C-11/95	11	**21**	12	13
C072	A	150	5	28.5	85.0	106	C-11/95	11	10	12	13
C073	A	220	5	28.5	85.0	106	C-11/95	11	10	12	13
C074	**B**	165	5	28.5	85.0	107	C-11/95	11	**21**	12	13
C075	A	140	5	28.5	85.0	107	C-11/95	11	10	12	13
C076	A	200	5	28.5	85.0	107	C-11/95	11	10	12	13
C077	A	145	5	28.5	85.0	107	C-11/95	11	10	12	13
C078	A	40	5	28.5	85.0	108	C-11/95	11	10	12	13
R001	A	95	1	26.5	96.5	019	R-07/95	11	10	12	13
R002	**D**	75	1	26.5	96.5	019	R-07/95	**22**	10	12	13
R003	**B**	120	1	26.5	96.5	019	R-07/95	11	**21**	12	13
R004	A	135	1	26.0	97.0	021	R-07/95	11	10	12	13
R005	A	115	1	26.0	97.0	021	R-07/95	11	10	12	13
R006	A	90	1	26.5	96.5	019	R-07/95	11	10	12	13
R007	A	65	1	26.5	96.5	019	R-07/95	11	10	12	13
R008	A	140	1	26.0	97.0	022	R-07/95	11	10	12	13
R009	A	145	1	26.0	97.0	022	R-07/95	11	10	12	13
R010	A	60	1	27.0	97.0	052	R-07/95	11	10	12	13
R011	**B**	140	1	27.0	97.0	052	R-07/95	11	**21**	12	13
R012	A	105	1	27.0	97.0	052	R-07/95	11	10	12	13
R013	A	150	1	27.5	97.0	057	R-07/95	11	10	12	13
R014	A	120	1	27.5	96.5	069	R-07/95	11	10	12	13
R015	A	60	1	27.5	96.5	069	R-07/95	11	10	12	13
R016	A	110	1	27.5	96.5	069	R-07/95	11	10	12	13
R017	**B**	135	1	27.5	96.5	069	R-07/95	11	**21**	12	13
R018	A	95	1	27.5	96.5	069	R-07/95	11	10	12	13
R019	A	85	1	28.0	96.5	076	R-07/95	11	10	12	13
R020	A	130	1	28.0	96.0	078	R-07/95	11	10	12	13
R021	**B**	165	1	28.0	96.0	078	R-07/95	11	**21**	12	13
R022	A	115	1	28.0	96.0	091	R-07/95	11	10	12	13
R023	A	130	1	28.0	96.0	092	R-07/95	11	10	12	13
R024	**D**	95	1	28.0	96.0	092	R-07/95	**22**	10	12	13
R025	A	90	1	28.0	96.0	090	R-07/95	11	10	12	13
R026	A	85	1	28.0	96.0	090	R-07/95	11	10	12	13
R027	A	130	1	28.0	96.5	072	R-07/95	11	10	12	13
R028	A	150	2	28.5	95.5	098	R-07/95	11	10	12	13
R029	A	135	2	28.5	95.5	098	R-07/95	11	10	12	13
R030	A	50	1	26.0	96.5	015	R-07/95	11	10	12	13
R031	A	50	1	26.0	96.5	015	R-07/95	11	10	12	13
R032	A	45	1	26.0	96.5	015	R-07/95	11	10	12	13
R033	A	100	1	27.5	96.5	070	R-07/95	11	10	12	13
R034	A	80	1	27.5	96.5	070	R-07/95	11	10	12	13
R035	**B**	135	1	27.5	96.5	070	R-07/95	11	**21**	12	13
R036	**B**	45	2	28.0	94.5	106	R-07/95	11	**21**	12	13
R037	A	110	2	28.0	94.5	118	R-07/95	11	10	12	13
R038	A	90	2	28.0	94.5	118	R-07/95	11	10	12	13
R039	**B**	105	2	28.0	94.5	118	R-07/95	11	**21**	12	13
R040	**B**	90	2	28.0	94.5	118	R-07/95	11	**21**	12	13
R041	A	55	2	28.0	93.5	124	R-07/95	11	10	12	13
R042	A	155	2	28.5	93.5	128	R-07/95	11	10	12	13
R043	A	160	2	28.5	93.5	135	R-07/95	11	10	12	13

(Table C-2 continued)

ID	Hap	Size	Unit	°N	°W	Sta	Cruise	□	▲	O	■
R044	**B**	130	2	28.5	93.5	135	R-07/95	11	**21**	12	13
R045	**B**	175	2	28.5	93.5	137	R-07/95	11	**21**	12	13
R046	**G**	125	2	28.5	93.0	160	R-07/95	11	10	12	**32**
R047	**B**	65	3	28.5	92.0	177	R-07/95	11	**21**	12	13
R048	A	140	3	28.5	92.0	177	R-07/95	11	10	12	13
R049	A	160	3	28.5	92.0	177	R-07/95	11	10	12	13
R050	A	130	3	28.5	91.5	178	R-07/95	11	10	12	13
R051	**B**	125	3	28.5	91.5	178	R-07/95	11	**21**	12	13
R052	A	125	3	29.0	92.0	181	R-07/95	11	10	12	13
R053	A	125	3	28.5	92.5	187	R-07/95	11	10	12	13
R054	A	135	3	28.5	92.0	190	R-07/95	11	10	12	13
R055	**F**	150	3	28.5	91.5	193	R-07/95	11	10	12	**22**
R056	**D**	95	3	28.0	91.5	195	R-07/95	**22**	10	12	13
R057	**B**	60	3	28.5	91.5	198	R-07/95	11	**21**	12	13
R058	A	60	3	28.5	91.5	198	R-07/95	11	10	12	13
R059	**B**	95	3	28.5	90.0	217	R-07/95	11	**21**	12	13
R061	A	100	3	28.5	90.0	217	R-07/95	11	10	12	13
R062	A	125	3	28.5	90.5	210	R-07/95	11	10	12	13
R063	**B**	–	3	28.5	90.5	210	R-07/95	11	**21**	12	13
S001	A	–	3	–	–	*	S-07/93	11	10	12	13
S002	A	–	3	–	–	*	S-07/93	11	10	12	13
S006	A	–	3	–	–	*	S-07/93	11	10	12	13
S007	A	–	3	–	–	*	S-07/93	11	10	12	13
S008	**C**	–	3	–	–	*	S-07/93	11	10	**21**	**22**
S009	A	–	3	–	–	*	S-07/93	11	10	12	13
S010	A	–	3	–	–	*	S-07/93	11	10	12	13
S011	**B**	195	4	30.0	88.5	095	S-07/93	11	**21**	12	13
S012	A	150	4	29.5	88.5	100	S-07/93	11	10	12	13
S013	A	45	4	29.5	88.5	344	S-07/93	11	10	nd	nd
S014	A	70	4	29.5	88.5	344	S-07/93	11	10	12	13
S015	A	40	4	29.0	88.5	343	S-07/93	11	10	12	13
S016	A	85	4	29.5	88.5	100	S-07/93	11	10	12	13
S017	A	115	4	29.0	88.5	345	S-07/93	11	10	12	13
S018	**C**	100	4	29.0	88.5	345	S-07/93	11	10	**21**	**22**
S019	A	90	4	29.0	88.5	345	S-07/93	11	10	12	13
S020	A	210	4	30.0	88.5	095	S-07/93	11	10	12	13
S021	A	195	2	28.0	94.5	210	S-07/93	11	10	12	13
S022	A	125	3	28.5	92.0	289	S-07/93	11	10	12	13
S023	A	80	4	29.0	88.5	345	S-07/93	11	10	12	13
S024	A	50	4	29.0	88.5	345	S-07/93	11	10	12	13
S025	**B**	60	4	29.0	88.5	345	S-07/93	11	**21**	12	13
S026	**H**	55	4	29.0	88.5	343	S-07/93	11	**21**	12	**22**
S027	**B**	50	4	29.0	88.5	343	S-07/93	11	**21**	12	13
S028	A	140	4	29.5	88.5	100	S-07/93	11	10	12	13

[1] Haplotype could be "C" or "F" due to missing restriction digest; assigned to C because that haplotype was much more common than F in sample unit 10.

[2] Haplotype could be "C" or "F" due to missing restriction digest; randomly assigned to F because either was as likely (total of four squid in sample unit 5 with these haplotypes).

* Exact locations of the trawl stations for these squid from sample unit 3 were unknown.

Table C-3. *Loligo plei* summary data: ID – squid identification number; Hap – mtDNA CO-I PCR-RFLP haplotype; Size – mantle length (nearest 5 mm); Unit – sample unit as described in Fig. 3; °N – latitude; °W – longitude; Sta – Station in original cruise data; Cruise (Table C-1). Restriction enzymes were *Ase*I (▣), *Hsp*92II (▼), *Msp*I (○). See text of Appendix C (or Figure 5) for interpreting restriction data. Bold-faced entries highlight Haplotypes B-K and "non-Haplotype A" restriction patterns.

ID	Hap	Size	Unit	°N	°W	Sta	Cruise	▣	▼	○
A033	A	125	10	35.5	75.5	037	A-10/96	11	11	11
A102	A	110	10	36.0	75.5	039	A-10/96	11	11	11
A401	A	140	10	36.0	75.5	038	A-10/96	11	11	11
A402	A	160	10	36.0	75.5	038	A-10/96	11	11	11
A403	A	160	10	36.0	75.5	038	A-10/96	11	11	11
B001	A	100	10	33.5	79.0	037	B-05/97	11	11	11
B002	A	250	9	31.0	81.0	071	B-05/97	11	11	11
B003	**C**	130	9	31.0	81.0	071	B-05/97	**22**	11	11
B004	**C**	130	9	31.0	81.0	071	B-05/97	**22**	11	11
B005	A	215	9	31.0	81.0	071	B-05/97	11	11	11
B006	**C**	140	9	31.0	81.0	071	B-05/97	**22**	11	11
B007	A	120	9	31.0	81.0	071	B-05/97	11	11	11
B008	A	120	9	31.0	81.0	071	B-05/97	11	11	11
B009	A	95	9	31.5	81.0	060	B-05/97	11	11	11
B010	A	115	9	31.5	81.0	060	B-05/97	11	11	11
B011	A	115	9	31.5	81.0	060	B-05/97	11	11	11
B012	A	185	9	31.5	81.0	060	B-05/97	11	11	11
B013	A	145	9	31.5	81.0	060	B-05/97	11	11	11
B014	**C**	115	9	31.5	81.0	060	B-05/97	**22**	11	11
B015	A	100	9	31.5	81.0	060	B-05/97	11	11	11
B016	A	100	9	31.5	81.0	060	B-05/97	11	11	11
B017	**C**	95	9	31.5	81.0	060	B-05/97	**22**	11	11
B018	A	150	10	32.5	80.0	054	B-05/97	11	11	11
B019	A	85	10	32.5	80.0	054	B-05/97	11	11	11
B020	**C**	75	10	32.5	80.0	054	B-05/97	**22**	11	11
B021	A	105	10	32.5	80.0	054	B-05/97	11	11	11
B022	**C**	125	10	32.5	80.0	051	B-05/97	**22**	11	11
B023	**C**	140	10	32.5	80.0	051	B-05/97	**22**	11	11
B024	**C**	115	10	32.5	80.0	051	B-05/97	**22**	11	11
B025	A	100	8	29.5	81.0	194	B-05/97	11	11	11
B026	A	85	8	29.5	81.0	194	B-05/97	11	11	11
B027	**C**	95	8	29.5	81.0	194	B-05/97	**22**	11	11
B028	A	110	8	29.5	81.0	194	B-05/97	11	11	11
B029	A	105	8	29.5	81.0	194	B-05/97	11	11	11
B030	A	95	8	29.5	81.0	194	B-05/97	11	11	11
B031	A	95	8	29.5	81.0	194	B-05/97	11	11	11
B032	A	140	9	31.0	81.0	069	B-05/97	11	11	11
B033	A	165	9	31.0	81.0	069	B-05/97	11	11	11
B034	A	135	9	31.0	81.0	069	B-05/97	11	11	11
B036	**C**	195	9	31.0	81.0	069	B-05/97	**22**	11	11
B037	**C**	175	9	31.0	81.0	069	B-05/97	**22**	11	11
B038	A	165	9	31.0	81.0	069	B-05/97	11	11	11
B039	A	95	10	32.5	80.5	121	B-05/97	11	11	11
B040	A	125	10	32.5	80.5	121	B-05/97	11	11	11
B041	**D**	110	10	32.5	80.5	121	B-05/97	**30**	11	11
B042	**B**	70	10	32.5	80.5	121	B-05/97	11	**20**	11
B043	**C**	85	10	32.5	80.5	121	B-05/97	**22**	11	11

(Table C-3 continued)

ID	Hap	Size	Unit	°N	°W	Sta	Cruise	▣	▼	O
B044	A	90	10	32.5	80.5	121	B-05/97	11	11	11
B045	A	95	10	32.5	80.5	121	B-05/97	11	11	11
B046	**C**	105	9	31.5	81.0	064	B-05/97	**22**	11	11
B047	A	140	9	31.5	81.0	064	B-05/97	11	11	11
B048	A	110	9	31.5	81.0	064	B-05/97	11	11	11
B049	**H**	95	9	31.5	81.0	064	B-05/97	**22**	**20**	11
B051	A	120	9	31.5	81.0	064	B-05/97	11	11	11
B052	**C**	130	9	31.5	81.0	064	B-05/97	**22**	11	11
B053	A	145	8	30.5	81.5	204	B-05/97	11	11	11
B054	A	170	8	30.5	81.5	204	B-05/97	11	11	11
B055	A	130	8	30.5	81.5	204	B-05/97	11	11	11
B056	A	160	8	30.5	81.5	204	B-05/97	11	11	11
B057	**C**	170	8	30.5	81.5	204	B-05/97	**22**	11	11
B058	A	100	8	30.5	81.5	204	B-05/97	11	11	11
B061	**C**	65	10	33.0	79.5	012	B-05/97	**22**	11	11
B064	A	90	9	32.0	80.5	115	B-05/97	11	11	11
B065	A	90	9	32.0	80.5	115	B-05/97	11	11	11
B066	A	90	9	32.0	80.5	115	B-05/97	11	11	11
B067	A	100	9	32.0	80.5	115	B-05/97	11	11	11
B068	A	115	9	32.0	80.5	115	B-05/97	11	11	11
B069	**C**	95	9	32.0	80.5	115	B-05/97	**22**	11	11
B070	A	80	8	29.5	81.0	186	B-05/97	11	11	11
B071	A	95	8	29.5	81.0	186	B-05/97	11	11	11
B072	A	100	8	29.5	81.0	186	B-05/97	11	11	11
B073	A	85	8	29.5	81.0	186	B-05/97	11	11	11
B074	A	100	8	29.5	81.0	186	B-05/97	11	11	11
B075	A	115	8	30.0	81.0	202	B-05/97	11	11	11
B076	A	135	8	30.0	81.0	202	B-05/97	11	11	11
B077	A	120	8	30.0	81.0	202	B-05/97	11	11	11
B078	**C**	125	8	30.0	81.0	202	B-05/97	**22**	11	11
B079	A	115	8	30.0	81.0	202	B-05/97	11	11	11
B080	**C**	130	8	30.0	81.0	202	B-05/97	**22**	11	11
B081	A	275	9	32.0	80.5	205	B-05/97	11	11	11
B082	**I**	115	9	32.0	80.5	205	B-05/97	**42**	11	11
B083	**C**	140	9	32.0	80.5	205	B-05/97	**22**	11	11
B084	A	145	9	32.0	80.5	205	B-05/97	11	11	11
B085	A	130	9	32.0	80.5	205	B-05/97	11	11	11
B086	A	110	10	32.5	80.0	055	B-05/97	11	11	11
B087	**C**	110	10	32.5	80.0	055	B-05/97	**22**	11	11
B088	A	95	10	32.5	80.0	055	B-05/97	11	11	11
B089	A	95	10	32.5	80.0	055	B-05/97	11	11	11
B090	A	130	10	32.5	80.0	055	B-05/97	11	11	11
B091	A	135	10	32.5	80.5	121	B-05/97	11	11	11
B092	**C**	95	10	32.5	80.5	121	B-05/97	**22**	11	11
B094	**C**	85	10	32.5	80.5	121	B-05/97	**22**	11	11
B095	A	95	10	32.5	80.5	121	B-05/97	11	11	11
B111	A	100	10	32.5	80.0	055	B-05/97	11	11	11
B112	A	125	10	32.5	80.0	055	B-05/97	11	11	11
B113	A	125	10	32.5	80.0	055	B-05/97	11	11	11
B114	**C**	95	10	32.5	80.0	055	B-05/97	**22**	11	11
B115	A	100	10	32.5	80.0	055	B-05/97	11	11	11
B116	A	110	10	32.5	80.0	055	B-05/97	11	11	11
B117	A	95	10	32.5	80.0	055	B-05/97	11	11	11

(Table C-3 continued)

ID	Hap	Size	Unit	°N	°W	Sta	Cruise	◙	▼	○
B118	A	110	10	32.5	80.0	055	B-05/97	11	11	11
B119	**C**	80	10	32.5	80.0	055	B-05/97	**22**	11	11
B120	**C**	90	10	32.5	80.0	055	B-05/97	**22**	11	11
B121	A	85	10	32.5	80.0	055	B-05/97	11	11	11
B126	A	120	9	32.0	80.5	205	B-05/97	11	11	11
B127	A	140	9	32.0	80.5	205	B-05/97	11	11	11
B128	A	150	9	32.0	80.5	205	B-05/97	11	11	11
B129	A	130	9	32.0	80.5	205	B-05/97	11	11	11
B130	**F**	150	9	32.0	80.5	205	B-05/97	11	11	**22**
B132	A	–	9	31.0	81.0	069	B-05/97	11	11	11
B133	A	–	9	31.0	81.0	069	B-05/97	11	11	11
B134	A	–	9	31.0	81.0	069	B-05/97	11	11	11
B135	A	–	9	31.0	81.0	069	B-05/97	11	11	11
B136	A	100	8	29.5	81.0	186	B-05/97	11	11	11
B137	A	125	9	32.0	80.5	205	B-05/97	11	11	11
B138	A	95	8	29.5	81.0	186	B-05/97	11	11	11
B139	A	75	8	29.5	81.0	186	B-05/97	11	11	11
B140	A	115	8	29.5	81.0	186	B-05/97	11	11	11
B141	**C**	95	8	29.5	81.0	186	B-05/97	**22**	11	11
B142	A	95	8	29.5	81.0	186	B-05/97	11	11	11
B143	A	100	8	29.5	81.0	194	B-05/97	11	11	11
B144	**C**	100	8	29.5	81.0	194	B-05/97	**22**	11	11
B145	**C**	120	8	29.5	81.0	194	B-05/97	**22**	11	11
B146	**J**	95	8	29.5	81.0	194	B-05/97	**22**	11	**32**
B147	A	100	8	29.5	81.0	194	B-05/97	11	11	11
B148	**B**	90	9	31.5	81.0	064	B-05/97	11	**20**	11
B149	A	95	9	31.5	81.0	064	B-05/97	11	11	11
B150	A	100	9	31.5	81.0	064	B-05/97	11	11	11
B151	A	115	9	31.5	81.0	064	B-05/97	11	11	11
B152	A	100	9	31.5	81.0	064	B-05/97	11	11	11
B153	A	95	9	32.0	80.5	115	B-05/97	11	11	11
B154	A	90	9	32.0	80.5	115	B-05/97	11	11	11
B155	**C**	90	9	32.0	80.5	115	B-05/97	**22**	11	11
B156	A	95	9	32.0	80.5	115	B-05/97	11	11	11
B157	A	100	9	32.0	80.5	115	B-05/97	11	11	11
B158	A	80	9	32.0	80.5	115	B-05/97	11	11	11
B159	**C**	125	9	31.0	81.0	071	B-05/97	**22**	11	11
B160	A	210	9	31.0	81.0	071	B-05/97	11	11	11
B161	A	195	9	31.0	81.0	071	B-05/97	11	11	11
B162	**B**	125	9	31.0	81.0	071	B-05/97	11	**20**	11
B163	**C**	110	9	31.0	81.0	071	B-05/97	**22**	11	11
B164	**C**	135	9	31.0	81.0	071	B-05/97	**22**	11	11
B165	A	125	9	31.0	81.0	071	B-05/97	11	11	11
B166	**C**	125	9	31.0	81.0	071	B-05/97	**22**	11	11
B167	A	145	8	30.5	81.5	204	B-05/97	11	11	11
B168	**C**	165	8	30.5	81.5	204	B-05/97	**22**	11	11
B169	A	100	8	30.5	81.5	204	B-05/97	11	11	11
B170	A	145	8	30.5	81.5	204	B-05/97	11	11	11
B171	**C**	120	8	30.0	81.0	202	B-05/97	**22**	11	11
B172	A	130	8	30.0	81.0	202	B-05/97	11	11	11
B173	A	125	8	30.0	81.0	202	B-05/97	11	11	11
B174	A	110	8	30.0	81.0	202	B-05/97	11	11	11
B175	A	100	8	30.0	81.0	202	B-05/97	11	11	11

(Table C-3 continued)

ID	Hap	Size	Unit	°N	°W	Sta	Cruise	◙	▼	O
B176	A	115	8	30.0	81.0	202	B-05/97	11	11	11
B177	A	80	10	32.5	80.5	121	B-05/97	11	11	11
B178	A	85	10	32.5	80.5	121	B-05/97	11	11	11
B179	A	80	10	32.5	80.5	121	B-05/97	11	11	11
B180	A	75	10	32.5	80.5	121	B-05/97	11	11	11
B181	A	75	10	32.5	80.5	121	B-05/97	11	11	11
B182	A	70	10	32.5	80.5	121	B-05/97	11	11	11
DT01	A	170	7	25.0	82.5	001	D-01/97	11	11	11
DT02	**C**	145	7	25.0	82.5	001	D-01/97	**22**	11	11
DT03	A	115	7	25.0	82.5	001	D-01/97	11	11	11
DT04	**C**	125	7	25.0	82.5	001	D-01/97	**22**	11	11
DT05	A	100	7	25.0	82.5	001	D-01/97	11	11	11
DT06	**D**	135	7	25.0	82.5	001	D-01/97	**30**	11	11
DT07	A	135	7	25.0	82.5	001	D-01/97	11	11	11
DT08	A	90	7	25.0	82.5	001	D-01/97	11	11	11
DT09	A	130	7	25.0	82.5	001	D-01/97	11	11	11
DT10	A	110	7	25.0	82.5	001	D-01/97	11	11	11
DT11	A	130	7	25.0	82.5	001	D-01/97	11	11	11
DT12	A	120	7	25.0	82.5	001	D-01/97	11	11	11
DT13	**C**	160	7	25.0	82.5	001	D-01/97	**22**	11	11
DT14	A	145	7	25.0	82.5	001	D-01/97	11	11	11
DT15	A	130	7	25.0	82.5	001	D-01/97	11	11	11
DT16	**C**	115	7	25.0	82.5	001	D-01/97	**22**	11	11
DT17	A	155	7	25.0	82.5	001	D-01/97	11	11	11
DT18	**C**	175	7	25.0	82.5	001	D-01/97	**22**	11	11
DT19	A	95	7	25.0	82.5	001	D-01/97	11	11	11
DT20	A	140	7	25.0	82.5	001	D-01/97	11	11	11
DT21	A	140	7	25.0	82.5	001	D-01/97	11	11	11
DT22	**C**	110	7	25.0	82.5	001	D-01/97	**22**	11	11
DT23	A	115	7	25.0	82.5	001	D-01/97	11	11	11
DT24	**C**	95	7	25.0	82.5	001	D-01/97	**22**	11	11
DT25	**C**	160	7	25.0	82.5	001	D-01/97	**22**	11	11
DT26	A	150	7	25.0	82.5	001	D-01/97	11	11	11
DT27	**E**	100	7	25.0	82.5	001	D-01/97	11	**32**	11
C201	A	120	7	29.0	84.0	103	C-11/95	11	11	11
C202	A	115	7	29.0	84.0	103	C-11/95	11	11	11
C203	A	80	7	29.0	84.0	103	C-11/95	11	11	11
C204	**C**	145	7	29.0	84.0	103	C-11/95	**22**	11	11
C205	A	115	7	29.0	84.0	103	C-11/95	11	11	11
C206	A	140	7	29.0	84.0	103	C-11/95	11	11	11
C207	**C**	220	7	29.0	84.0	102	C-11/95	**22**	11	11
C208	A	150	7	29.0	84.0	102	C-11/95	11	11	11
C209	A	190	7	29.0	84.0	102	C-11/95	11	11	11
C210	A	180	7	29.0	84.0	102	C-11/95	11	11	11
C211	**C**	35	6	30.0	86.5	088	C-11/95	**22**	11	11
C212	**C**	50	6	30.0	86.5	086	C-11/95	**22**	11	11
C213	A	70	6	30.0	86.5	089	C-11/95	11	11	11
C214	**C**	45	6	30.0	86.5	089	C-11/95	**22**	11	11
C215	A	35	6	30.0	86.5	089	C-11/95	11	11	11
C216	**C**	65	6	30.0	86.5	089	C-11/95	**22**	11	11
C217	**C**	50	6	30.0	86.5	089	C-11/95	**22**	11	11
C218	A	45	6	30.0	86.5	089	C-11/95	11	11	11
C219	**C**	55	6	30.0	86.5	089	C-11/95	**22**	11	11

(Table C-3 continued)

ID	Hap	Size	Unit	°N	°W	Sta	Cruise	⊡	▼	O
C220	**C**	50	6	30.0	86.5	089	C-11/95	**22**	11	11
C221	A	45	6	30.0	86.5	089	C-11/95	11	11	11
C222	A	40	6	30.0	86.5	089	C-11/95	11	11	11
C223	A	105	6	30.0	86.5	091	C-11/95	11	11	11
C224	**C**	115	6	30.0	86.5	091	C-11/95	**22**	11	11
C225	**C**	105	6	30.0	86.5	091	C-11/95	**22**	11	11
C226	A	95	6	30.0	86.5	091	C-11/95	11	11	11
C227	A	85	6	30.0	86.5	091	C-11/95	11	11	11
C228	**K**	85	6	30.0	86.5	091	C-11/95	**53**	11	11
C229	**C**	90	6	30.0	86.5	091	C-11/95	**22**	11	11
C230	**C**	75	6	30.0	86.5	091	C-11/95	**22**	11	11
C231	A	65	6	30.0	86.5	091	C-11/95	11	11	11
C232	**C**	70	6	30.0	86.5	091	C-11/95	**22**	11	11
C233	**C**	50	6	30.0	86.5	091	C-11/95	**22**	11	11
C234	A	40	6	30.0	86.5	091	C-11/95	11	11	11
C235	A	40	6	30.0	86.5	091	C-11/95	11	11	11
C236	A	50	6	30.0	86.5	091	C-11/95	11	11	11
C237	A	30	6	29.5	88.0	078	C-11/95	11	11	11
C238	A	35	6	29.5	88.0	078	C-11/95	11	11	11
C239	A	40	6	29.5	88.0	078	C-11/95	11	11	11
C240	**B**	35	6	29.5	88.0	078	C-11/95	11	**20**	11
C241	A	45	6	29.5	88.0	078	C-11/95	11	11	11
C242	**B**	30	6	29.5	88.0	078	C-11/95	11	**20**	11
C243	**I**	30	6	29.5	88.0	078	C-11/95	**42**	11	11
C244	A	60	6	29.5	88.0	075	C-11/95	11	11	11
C245	A	30	6	30.0	88.0	070	C-11/95	11	11	11
C246	A	25	6	30.0	88.0	070	C-11/95	11	11	11
C247	**B**	30	6	30.0	88.0	070	C-11/95	11	**20**	11
C248	A	25	6	30.0	88.0	070	C-11/95	11	11	11
C249	**C**	30	6	30.0	88.0	070	C-11/95	**22**	11	11
C250	**B**	35	6	30.0	88.0	070	C-11/95	11	**20**	11
C251	A	30	6	30.0	88.0	070	C-11/95	11	11	11
C252	A	15	6	30.0	88.0	070	C-11/95	11	11	11
C253	A	30	6	30.0	88.0	071	C-11/95	11	11	11
C254	A	30	6	30.0	88.0	071	C-11/95	11	11	11
C255	A	30	6	30.0	88.0	071	C-11/95	11	11	11
C256	A	25	6	30.0	88.0	071	C-11/95	11	11	11
C257	**C**	25	6	30.0	88.0	071	C-11/95	**22**	11	11
C258	A	20	6	30.0	88.0	071	C-11/95	11	11	11
C259	**C**	105	6	29.5	88.0	074	C-11/95	**22**	11	11
C260	A	20	6	29.5	88.0	074	C-11/95	11	11	11
C261	A	45	6	29.5	88.0	074	C-11/95	11	11	11
C262	A	40	6	29.5	88.0	074	C-11/95	11	11	11
C263	A	20	6	29.5	88.0	074	C-11/95	11	11	11
C264	A	35	6	29.5	88.0	074	C-11/95	11	11	11
C265	**B**	40	6	29.5	88.0	074	C-11/95	11	**20**	11
C266	A	25	6	29.5	88.0	074	C-11/95	11	11	11
C267	**B**	75	6	30.0	88.5	064	C-11/95	11	**20**	11
C268	A	95	6	30.0	87.0	079	C-11/95	11	11	11
C269	**C**	50	6	30.0	87.0	079	C-11/95	**22**	11	11
C270	**C**	55	6	30.0	87.0	079	C-11/95	**22**	11	11
C271	**C**	45	6	30.0	87.0	079	C-11/95	**22**	11	11

(Table C-3 continued)

ID	Hap	Size	Unit	°N	°W	Sta	Cruise	⊡	▼	O
C272	**C**	35	6	30.0	87.0	079	C-11/95	**22**	11	11
C273	**C**	40	6	30.0	87.0	079	C-11/95	**22**	11	11
C274	**C**	40	6	30.0	87.0	079	C-11/95	**22**	11	11
C275	A	30	6	30.0	87.0	079	C-11/95	11	11	11
C276	A	35	6	30.0	87.0	079	C-11/95	11	11	11
C277	**B**	35	6	30.0	87.0	079	C-11/95	11	**20**	11
C278	**B**	45	6	29.5	88.0	077	C-11/95	11	**20**	11
C279	A	40	6	29.5	88.0	077	C-11/95	11	11	11
C280	A	40	6	29.5	88.0	077	C-11/95	11	11	11
C281	A	35	6	29.5	88.0	077	C-11/95	11	11	11
C282	A	35	6	29.5	88.0	077	C-11/95	11	11	11
C283	A	50	6	29.5	88.0	077	C-11/95	11	11	11
C284	A	35	6	29.5	88.0	077	C-11/95	11	11	11
C285	**C**	40	6	29.5	88.0	077	C-11/95	**22**	11	11
C286	A	140	6	30.0	87.0	080	C-11/95	11	11	11
C287	A	140	6	30.0	87.0	080	C-11/95	11	11	11
C288	A	125	6	30.0	87.0	080	C-11/95	11	11	11
C289	**C**	105	6	30.0	87.0	080	C-11/95	**22**	11	11
C290	**C**	100	6	30.0	87.0	080	C-11/95	**22**	11	11
C291	A	90	6	30.0	87.0	080	C-11/95	11	11	11
C292	A	100	6	30.0	87.0	080	C-11/95	11	11	11
C293	A	85	6	30.0	87.0	080	C-11/95	11	11	11
C294	**C**	105	6	30.0	87.0	080	C-11/95	**22**	11	11
C295	A	100	6	30.0	87.0	080	C-11/95	11	11	11
C296	A	30	4	29.0	92.5	044	C-11/95	11	11	11
C297	A	40	4	29.0	92.5	044	C-11/95	11	11	11
C298	A	40	4	29.0	92.5	044	C-11/95	11	11	11
C299	A	45	4	29.0	92.5	044	C-11/95	11	11	11
C300	A	50	4	29.0	92.5	044	C-11/95	11	11	11
C301	**B**	65	4	29.0	92.5	044	C-11/95	11	**20**	11
C302	A	30	3	28.5	94.5	022	C-11/95	11	11	11
C303	**C**	30	3	28.5	94.5	022	C-11/95	**22**	11	11
C304	A	35	3	28.5	94.5	022	C-11/95	11	11	11
C305	**B**	25	3	28.5	94.5	022	C-11/95	11	**20**	11
C306	**B**	35	3	28.5	94.5	022	C-11/95	11	**20**	11
C307	**C**	35	3	28.5	94.5	022	C-11/95	**22**	11	11
C308	**B**	30	3	28.5	94.5	022	C-11/95	11	**20**	11
C309	A	30	3	28.5	94.5	022	C-11/95	11	11	11
C310	A	30	3	28.5	94.5	022	C-11/95	11	11	11
C311	**C**	30	3	28.5	94.5	022	C-11/95	**22**	11	11
C312	**B**	30	3	28.5	94.5	020	C-11/95	11	**20**	11
C313	**B**	25	3	28.5	94.5	020	C-11/95	11	**20**	11
C314	**B**	30	3	28.5	94.5	020	C-11/95	11	**20**	11
C315	**B**	35	3	28.5	94.5	020	C-11/95	11	**20**	11
C316	A	25	3	28.5	94.5	020	C-11/95	11	11	11
C317	**B**	25	3	28.5	94.5	020	C-11/95	11	**20**	11
C318	**H**	25	3	28.5	94.5	020	C-11/95	**22**	**20**	11
C319	**B**	30	3	28.5	94.5	020	C-11/95	11	**20**	11
C320	**C**	35	3	28.5	94.5	020	C-11/95	**22**	11	11
C321	A	35	3	28.5	94.5	020	C-11/95	11	11	11
C322	A	25	5	28.5	91.5	053	C-11/95	11	11	11
C323	**C**	25	5	28.5	91.5	053	C-11/95	**22**	11	11

(Table C-3 continued)

ID	Hap	Size	Unit	°N	°W	Sta	Cruise	▣	▼	O
C324	**B**	20	5	28.5	91.5	053	C-11/95	11	**20**	11
C325	A	25	5	28.5	91.5	053	C-11/95	11	11	11
C326	**B**	25	5	28.5	91.5	053	C-11/95	11	**20**	11
C327	**C**	25	5	28.5	91.5	053	C-11/95	**22**	11	11
C328	A	25	5	28.5	91.5	053	C-11/95	11	11	11
C329	**C**	20	5	28.5	91.5	053	C-11/95	**22**	11	11
C330	**C**	20	5	28.5	91.5	053	C-11/95	**22**	11	11
C331	A	20	5	28.5	91.5	053	C-11/95	11	11	11
C332	A	55	2	27.5	97.0	010	C-11/95	11	11	11
C333	**B**	30	2	27.5	97.0	010	C-11/95	11	**20**	11
C334	A	45	2	27.5	97.0	010	C-11/95	11	11	11
C335	**B**	55	2	27.5	97.0	010	C-11/95	11	**20**	11
C336	A	30	2	27.5	97.0	010	C-11/95	11	11	11
C337	A	25	2	27.5	97.0	010	C-11/95	11	11	11
C338	**B**	30	2	27.5	97.0	010	C-11/95	11	**20**	11
C339	**B**	30	2	27.5	97.0	010	C-11/95	11	**20**	11
C340	A	25	2	27.5	97.0	010	C-11/95	11	11	11
R201	**C**	90	2	28.0	97.0	065	R-07/95	**22**	11	11
R202	**B**	70	2	28.0	97.0	066	R-07/95	11	**20**	11
R203	A	90	2	28.0	96.5	071	R-07/95	11	11	11
R204	A	55	2	28.0	96.5	071	R-07/95	11	11	11
R205	**B**	55	2	28.0	96.5	071	R-07/95	11	**20**	11
R206	**C**	100	2	28.0	96.5	080	R-07/95	**22**	11	11
R207	**B**	80	2	28.0	96.0	091	R-07/95	11	**20**	11
R208	**B**	70	2	28.5	95.5	100	R-07/95	11	**20**	11
R209	**B**	45	4	28.5	92.5	162	R-07/95	11	**20**	11
R210	**B**	50	4	28.5	92.5	162	R-07/95	11	**20**	11
R211	**B**	45	4	28.5	92.5	162	R-07/95	11	**20**	11
R212	**B**	80	4	28.5	92.5	162	R-07/95	11	**20**	11
R213	**B**	75	4	28.5	92.5	162	R-07/95	11	**20**	11
R214	**C**	75	4	28.5	92.0	180	R-07/95	**22**	11	11
R215	**B**	55	4	28.5	92.0	180	R-07/95	11	**20**	11
R216	A	35	4	28.0	92.5	170	R-07/95	11	11	11
R217	**B**	60	4	28.5	92.0	190	R-07/95	11	**20**	11
R218	**C**	75	5	28.5	90.5	209	R-07/95	**22**	11	11
R219	**B**	50	4	28.5	92.0	180	R-07/95	11	**20**	11
R220	**C**	55	5	29.0	90.0	218	R-07/95	**22**	11	11
R221	**B**	50	5	29.0	90.0	218	R-07/95	11	**20**	11
R222	A	—	1	26.0	97.0	032	R-07/95	11	11	11
R223	A	—	1	26.0	97.0	032	R-07/95	11	11	11
R224	A	40	5	28.5	91.5	193	R-07/95	11	11	11
R225	A	50	5	28.5	91.5	193	R-07/95	11	11	11
R226	A	50	5	28.5	91.5	193	R-07/95	11	11	11
R227	**B**	45	5	28.5	91.5	193	R-07/95	11	**20**	11
R228	**B**	85	2	27.0	97.5	061	R-07/95	11	**20**	11
R229	**B**	90	2	27.0	97.5	061	R-07/95	11	**20**	11
R230	**B**	100	2	27.0	97.5	061	R-07/95	11	**20**	11
R231	**B**	85	2	27.0	97.5	061	R-07/95	11	**20**	11
R232	**B**	60	2	27.0	97.5	061	R-07/95	11	**20**	11
R233	**B**	80	2	27.0	97.5	061	R-07/95	11	**20**	11
R234	**B**	175	2	27.0	97.5	061	R-07/95	11	**20**	11
R235	**B**	155	2	27.0	97.5	061	R-07/95	11	**20**	11

(Table C-3 continued)

ID	Hap	Size	Unit	°N	°W	Sta	Cruise	▣	▼	O
R236	**B**	60	5	28.5	90.5	216	R-07/95	11	**20**	11
R237	**B**	65	5	28.5	90.5	216	R-07/95	11	**20**	11
R238	A	60	5	28.5	90.5	216	R-07/95	11	11	11
R239	**B**	70	5	28.5	90.5	216	R-07/95	11	**20**	11
R240	**C**	70	5	28.5	90.5	216	R-07/95	**22**	11	11
R241	**C**	85	5	28.5	90.5	216	R-07/95	**22**	11	11
R242	**B**	110	5	28.5	90.5	216	R-07/95	11	**20**	11
R243	**B**	100	5	28.5	90.5	216	R-07/95	11	**20**	11
R244	A	65	5	28.5	90.5	216	R-07/95	11	11	11
R245	A	95	5	28.5	90.5	216	R-07/95	11	11	11
R246	**B**	80	5	28.5	90.5	216	R-07/95	11	**20**	11
R247	**B**	100	5	28.5	90.5	216	R-07/95	11	**20**	11
R248	**B**	200	1	26.5	97.0	028	R-07/95	11	**20**	11
R249	**B**	175	1	26.5	97.0	028	R-07/95	11	**20**	11
R250	A	175	1	26.5	97.0	028	R-07/95	11	11	11
R251	**B**	160	1	26.5	97.0	028	R-07/95	11	**20**	11
R252	**B**	190	1	26.5	97.0	028	R-07/95	11	**20**	11
R253	**B**	175	1	26.5	97.0	028	R-07/95	11	**20**	11
R254	**B**	140	1	26.5	97.0	028	R-07/95	11	**20**	11
R255	A	170	1	26.5	97.0	028	R-07/95	11	11	11
R256	**B**	170	1	26.5	97.0	028	R-07/95	11	**20**	11
R257	**B**	160	1	26.5	97.0	028	R-07/95	11	**20**	11
R258	A	180	1	26.5	97.0	028	R-07/95	11	11	11
R259	**B**	180	1	26.5	97.0	028	R-07/95	11	**20**	11
R260	A	160	1	26.5	97.0	028	R-07/95	11	11	11
R261	**B**	160	1	26.5	97.0	028	R-07/95	11	**20**	11
R262	**B**	170	1	26.5	97.0	028	R-07/95	11	**20**	11
SL01	A	–	4	–	–	*	S-07/93	11	11	11
SL02	**B**	–	4	–	–	*	S-07/93	11	**20**	11
SL03	**C**	–	4	–	–	*	S-07/93	**22**	11	11
SL04	**B**	–	4	–	–	*	S-07/93	11	**20**	11
SL05	A	–	4	–	–	*	S-07/93	11	11	11
SL06	**B**	–	4	–	–	*	S-07/93	nd	**20**	11
SL07	**B**	–	4	–	–	*	S-07/93	11	**20**	11
SL08	**F**	45	2	28.0	96.0	169	S-07/93	11	11	**22**
SL09	**B**	40	2	28.0	95.5	185	S-07/93	11	**20**	11
SL10	**C**	50	2	28.0	95.5	185	S-07/93	**22**	11	11
SL11	A	45	4	28.5	93.0	264	S-07/93	11	11	11
SL12	**B**	55	4	28.5	92.5	257	S-07/93	11	**20**	11
SL13	**C**	80	4	28.5	92.5	257	S-07/93	**22**	11	11
SL14	**B**	40	1	26.0	96.5	109	S-07/93	11	**20**	11
SL15	**B**	55	1	26.0	96.5	109	S-07/93	11	**20**	nd
SL16	A	150	2	28.5	95.5	186	S-07/93	11	11	11
SL17	**B**	160	6	30.0	88.0	098	S-07/93	11	**20**	11
SL18	**B**	125	4	29.0	93.0	271	S-07/93	11	**20**	11
SL20	A	75	5	28.5	91.5	311	S-07/93	11	11	11
SL21	A	65	2	28.0	96.0	158	S-07/93	11	11	11
SL22	**B**	35	5	28.5	90.5	315	S-07/93	11	**20**	11
SL23	**B**	65	2	28.0	96.0	162	S-07/93	11	**20**	nd
SL24	**B**	60	5	28.5	91.5	295	S-07/93	11	**20**	11
SL25	**B**	55	4	28.5	92.0	287	S-07/93	11	**20**	11

(Table C-3 continued)

ID	Hap	Size	Unit	°N	°W	Sta	Cruise	◙	▼	O
SL26	**C**	55	4	28.5	92.0	287	S-07/93	**22**	11	11
SL27	**B**	50	1	26.0	96.5	109	S-07/93	11	**20**	nd
SL28	**B**	50	1	26.0	96.5	109	S-07/93	11	**20**	nd
SL29	**B**	50	1	26.0	96.5	109	S-07/93	11	**20**	11
SL30	**B**	45	1	26.0	96.5	109	S-07/93	11	**20**	nd
SL31	**B**	50	1	26.0	96.5	109	S-07/93	11	**20**	11
SL32	A	45	1	26.0	96.5	109	S-07/93	11	11	11
SL33	**B**	65	2	28.0	96.0	158	S-07/93	11	**20**	11
SL34	**C**	50	5	28.5	90.5	315	S-07/93	**22**	11	11
SL35	**B**	60	5	28.5	91.0	310	S-07/93	11	**20**	11
SL36	**B**	60	3	28.0	94.5	207	S-07/93	11	**20**	11
SL37	**B**	65	3	28.0	94.5	207	S-07/93	11	**20**	11
SL38	**B**	55	3	28.0	94.5	207	S-07/93	11	**20**	11
SL39	**C**	60	4	28.5	92.0	287	S-07/93	**22**	11	11
SL40	**C**	60	4	28.5	92.0	287	S-07/93	**22**	11	11
SL41	**B**	50	1	26.0	96.5	109	S-07/93	11	**20**	11
SL42	A	55	1	26.0	96.5	109	S-07/93	11	11	11
SL43	**G**	40	1	26.0	96.5	109	S-07/93	**42**	**20**	11

*** Exact locations of the trawl stations for these squid were unknown, but they were taken from either sample unit 4 or 5. Haplotype frequencies in these units were nearly identical both with and without these specimens, so all seven squid were placed into sample unit 4 to equalize sample sizes between the two sample units.**

Table C-4: PCR-RFLP haplotype frequency data (mtDNA CO-I) grouped by survey cruise for two species of *Loligo* squid. Refer to Table C-1 to determine the season and year of each survey based on the cruise designations. Sample sizes (*n*) and the approximate latitudinal (°N) or longitudinal (°W) zone in which the cruise occurred are noted.

A. *L. pealei* (longfin squid)

Cruise Information			Haplotype Frequency (i.e., the % of *n* for that cruise)											
Cruise[1]	*n*	Area	A	B	C	D	E	F	G	H	I	J	K	L	M
A-02/96	48	39-41°N	59	4	15	6	8	2	-	2	-	-	2	2	-
A-04/96	26	37-43°N	92	4	4	-	-	-	-	-	-	-	-	-	-
A-10/96	60	35-42°N	71	7	10	-	5	5	-	2	-	-	-	-	-
B-05/97	57	31-35°N	74	7	11	-	4	2	2	-	-	-	-	-	-
Cruise[2]	*n*	Area	A	B	C	D	E	F	G	H	I	J	K	L	M
S-07/93	25	88-92°W	76	12	8	-	-	-	-	4	-	-	-	-	-
R-07/95	62	90-97°W	67	24	-	5	-	2	2	-	-	-	-	-	-
C-11/95	78	84-94°W	65	27	-	4	-	1	-	-	1	1	-	-	1

B. *L. plei* (arrow squid)

Cruise Information			Haplotype Frequency (i.e., the % of *n* for that cruise)										
Cruise[3]	*n*	Area	A	B	C	D	E	F	G	H	I	J	K
B-05/97	160[a]	80-82°W	71	2	21	1	-	1	-	1	1	1	-
D-01/97	27	82.5°W	62	-	30	4	4	-	-	-	-	-	-
C-11/95	95	84-88°W	60	8	30	-	-	-	-	-	1	-	1
Cruise[4]	*n*	Area	A	B	C	D	E	F	G	H	I	J	K
S-07/93	22	90-93°W	23	50	27	-	-	-	-	-	-	-	-
R-07/95	29	90-93°W	24	59	17	-	-	-	-	-	-	-	-
C-11/95	16	90-93°W	56	19	25	-	-	-	-	-	-	-	-
S-07/93	20	94-97°W	15	70	5	-	-	5	5	-	-	-	-
R-07/95	33	94-97°W	24	70	6	-	-	-	-	-	-	-	-
C-11/95	29	94-97°W	38	45	14	-	-	-	-	3	-	-	-

[1] Atlantic Ocean population.
[2] Gulf of Mexico population.
[3] Eastern population.
[4] Western population.
[a] Includes five squid (all with Haplotype A) taken on Cruise A-10/96.

Table C-5. Specimens from which mtDNA CO-I sequences were obtained. Sequence designations are as in Table 4; specimen designations are as in Tables C-2 and C-3.

Loligo pealei		*Loligo plei*	
Sequence	Specimen	Sequence	Specimen
A_1	R001	A_1	R216
A_2	B245	A_2	B001
A_3	A128	B_1	B148
B_1	A025	B_2	B042
B_2	R011	B_3	B162
C_1	A052	B_4	C278
C_2	S008	B_5	C306
C_3	S018	C_1	B144
D_1	A073	C_2	B020
D_2	R002	C_3	C307
E	A055	D_1	DT06
F_1	A127	D_2	B041
F_2	C070	E	D027
G_1	RO46	F_1	SL08
G_2	B035	F_2	B130
H_1	A080	G	SL43
H_2	A123	H_1	C318
H_3	S026	H_2	B049
I	C007	I_1	C243
J	C039	I_2	B082
K	A092	J	B146
L	A063	K	C228
M	C050		

APPENDIX D: DNA EXTRACTIONS

DNA was initially extracted by a simple salting-out procedure in which approximately 20 mg of tissue were ground in 500 µl of preheated (55°C) extraction buffer [STE (100 mM NaCl, 10 mM Tris, 5 mM EDTA, pH 8.0) with 0.2% SDS (sodium dodecyl sulfate) and 100 µg/ml Proteinase-K (> 30 u/mg from Amresco, Solon, OH)]. To inactivate DNases, the extraction was maintained at 55°C for more than 30 minutes. After the addition of 50 µl 5M NaCl and centrifugation (5 min at 16,000 x g), the DNA in the supernatant was ethanol precipitated in a clean tube. The salt-extraction method produced adequate quantities of DNA; however, the DNA degraded rapidly during storage at -20°C and from freeze-thaw cycles. Thus, further extractions followed the protocol below (all times are approximate).

DNA from this extraction protocol was highly stable, even after multiple freeze-thaw cycles. A standard 1.7 ml microcentrifuge tube containing 270 µl of STE (0.2% SDS, 100 µg/ml Proteinase K, pH 8.0) was heated to 55°C in a hot block prior to adding about 10 – 20 mg of squid mantle tissue. The tissue was immediately crushed with an autoclaved pestle and returned to the hot block. The heated buffer, small tissue mass, and immediate crushing were all necessary to consistently produce high-quality DNA extractions. After incubation (5 – 30 min) with the Proteinase K, 30 µl sodium acetate (pH 5.2) were added to the tube and the contents were mixed by inversion. Cellular debris and salted-out proteins were then sedimented at 16,000 x g (3 min), and the supernatant was decanted into a 1.5 ml Phase Lock Gel-L (PLG) tube (5 Prime → 3 Prime, Boulder, CO).

The aqueous contents of the PLG tube were mixed by inversion with 10 units of RNase *A* (Amresco, Solon, OH). After incubation at room temperature (2 min), the extraction was purified by succeeding additions of 250 µl phenol (5 min), 250 µl phenol-chloroform-isoamyl alcohol (5 min), and 200 µl chloroform (2 min). At each addition, the tube was touch-vortexed to thoroughly emulsify the new organic solvents with the aqueous layer. In between additions,

the PLG tube was centrifuged at 16,000 x g (2 min) to ensure complete phase separation. The gel inside the PLG tube had a density intermediate to the aqueous and organic phases, so denatured proteins and lipids were sequestered in the organic phase underneath the gel layer. The gel layer was impervious to vortexing, so each new addition of organic solvent operated independently of the prior additions.

The aqueous supernatant was decanted into a standard 1.7 ml microcentrifuge tube containing 700 µl absolute ethanol, and the DNA was allowed to precipitate at room temperature (> 10 min). The DNA was pelleted at 16,000 x g (> 15 minutes), and the supernatants were discarded by decanting. The non-visible DNA pellets were washed twice with 70% ethanol (700 µl per wash), dried in a Speed-Vac at 65°C, and resuspended in 50 µl TE (10 mM Tris, 1 mM EDTA, pH 8.0). Electrophoresis of 5 µl of the DNA suspension in a 1% agarose gel indicated that most extractions were RNA-free and produced a total yield of 0.5 – 4.0 µg of high-molecular weight (>23 kb) DNA.

VITA

Scott William Herke was born in Vero Beach, Florida, on 29 March 1963. Within two months, his family moved from Florida to Baton Rouge, Louisiana. From an early age, he helped his father, Professor William Herbert Herke, with fisheries research projects in the coastal marshes of Louisiana. At the same time, he learned to appreciate the wealth of outdoor recreational opportunities that are available in Louisiana, including canoeing, fishing, and hunting. After graduating from Catholic High School in 1981, he attended Louisiana State University, Baton Rouge, where he completed a bachelor of science degree in forestry (with a wildlife option). In 1986, he left Baton Rouge to attend graduate school at the University of Maine in Orono, Maine. He completed his masters of science degree in zoology in 1988, and accepted a position with the United States Army Corps of Engineers in Waltham, Massachusetts. For the next five years under the jurisdiction of Section 404 of the Clean Water Act and Section 10 of the Rivers and Harbors Act of 1899, he processed applications from the public regarding requests to perform work in waters of the United States (including wetlands). On 16 May 1992, he married Linda Marie Heffernan after a courtship of nearly six years. In 1993, he and his wife moved to Baton Rouge, Louisiana, to attend graduate school at Louisiana State University. With his marriage still intact, he graduated with a degree of Doctor of Philosophy in the biological sciences in December 1999.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Scott William Herke

Major Field: Zoology

Title of Dissertation: Phylogeography of Two Loligo Squid (Cephalopoda: Myopsida) in the Gulf of Mexico and the Northwestern Atlantic Ocean

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

July 26, 1999